CHARACTERIZING SOIL PROPERTIES AND BIOGEOCHEMICAL PROCESSES IN A MOUNTAIN PEATLAND

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By

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ABSTRACT

Northern peatlands are important to the global carbon (C) and nitrogen (N) cycles. Peat profiles in Rocky Mountain areas commonly show complex stratigraphy with underlying and/or interbedded mineral sediments, referred to as stratified mineral horizons. Stratified mineral horizons usually have lower hydraulic conductivity and more electron acceptors, which influences biogeochemical processes. To study the effect of mineral sediments on pedological and biogeochemical processes, I conducted a field study and a microcosm study. The field study was located in a mountain peatland in the foothills of the Canadian Rocky Mountains with three different organic soil types: sedge peat/silty sediments/calcareous sediments (PMC), sedge peat/silty sediments/moss peat (PMP) and sedge peat/moss peat (PP). Soil samples were tested for spatial distribution of total organic C (TOC), total N (TN), pH, volumetric water content (θv), C and N cycling rates, C composition and microbial community structure. A microcosm study was designed to mimic climate warming conditions with four temperature-water table treatments: current temperature/current water table, higher temperature/current water table, current temperature/lower water table, and higher temperature/lower water table. In the microcosm study, PMC and PP soils were incubated for 28 days and tested for GHG emissions and concentrations, biogeochemical process rates, apparent enzyme activation energy (E_a) and bacterial community structure.

In the field study, results indicated mineral sediments mainly affect pedological and biogeochemical processes in subsurface peat rather than surface peat. Mineral sediments affected the spatial distributions of total organic C (TOC), total N (TN), pH and volumetric water content (θv) via elevating the pH adjacent to calcareous sediments and slowing water infiltration to lower depths. The pH and θv further affected TOC and TN distribution by regulating organic matter decomposition during the peatland's geomorphic history. At the same time, mineral sediments also affected C and N cycling processes, though depth had an even greater effect. The effect of mineral sediments on N cycling was mainly due to high pH from calcareous sediments, which promoted net nitrification but lowered net ammonification in the PMC. Moreover, mineral sediment mitigated the lag phase of N cycling in deeper layers. The effect of mineral sediments on C cycling was reflected in two aspects, geomorphic history and hydrological conditions. During the peatland's geomorphic history, mineral horizons promoted decomposition by

increasing pH and providing electron acceptors in overlying peat. Enhanced decomposition in the past resulted in more recalcitrant materials in peat at present. This, combined with physicochemical protection of C by mineral sediments, further restricted C mineralization in the PMP and PMC. Hydrologically, stratified mineral horizons slowed water infiltration and resulted in higher θv above the mineral horizon and lower θv below the mineral horizon. This restricted C mineralization in peat above mineral sediment and encouraged C mineralization in peat below mineral sediment in PMP. In addition, these factors also affected microbial community structure, with the highest Stress and Bacteria:Fungi ratio in peat above mineral sediment and different microbial community structure in peat below mineral sediments.

In the microcosm study, I found that high temperature increased GHG emission and GHG concentration – especially at depth – in most samples. Soil types affected CO_2 and N_2O concentrations from subsurface horizons: PP had higher CO_2 and N_2O than PMC. Importantly, N_2O concentration and production rates were affected by interaction of soil types and temperature near the water table: N_2O production in PP was more enhanced by high temperature. This was possibly because PP had greater labile C and lower pH. In addition, compared with PP, the E_a for N_2O generation in PMC was increased more by high temperature incubation and microbial community structures were quite different in the two soils, especially the lower relative abundance of copiotrophs in PMC.

Overall, the findings highlight that stratified mineral sediment affected spatial distribution of key soil properties, which influenced biogeochemical processes in this mountain peatland. Elevated pH due to calcareous sediment promoted nitrification and C mineralization. In addition, stratified mineral sediment affected θv, which then affected microbial community structure and C mineralization. Under a warming climate, compared with a continuous peat profile, peat with mineral sediments tends to have less labile C and higher pH, which could potentially result in less CO_2 and N_2O emission and mitigate N_2O production proximal to the water table.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ANOSIM	Similarity analysis
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
CCA	Canonical correspondence analysis
B:F	Bacteria to fungi ratio
CEC	Cation Electronic Conductivity
CT	Current temperature (15°C)
CW	Current water table (25 cm below surface)
DNA	Deoxyribonucleic acid
DP	Deep peat
E_a	Apparent enzyme activation energy
EC	Electronic conductivity
Eh	Redox potential
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatograph
GHG	Greenhouse gases
GP:GN	Gram positive bacteria to gram negative bacteria ratio
HT	Higher temperature (25°C)
LW	Lower water table (40 cm below surface)
MANOVA	Multivariate analysis of variance
MP	Middle peat
MRPP	Multi-Response Permutation Procedures
NMDS	Nonmetric multidimensional scaling
NPP	Net primary production
OTU	Operational taxonomic unit
PLFA	Phospholipid fatty acid
PMC	Sedge peat/silty mineral sediments/calcareous sediments
PMP	Sedge peat/silty mineral sediments/moss peat
PP	Sedge peat/moss peat
R_{amm}	Net ammonification rate
RC:LC	Recalcitrant C to labile C ratio
R_{nit}	Net nitrification rate
SIMPER	Similarity percentage
SOC	Soil organic carbon
SOM	Soil organic matter
SP	Surface peat

TN	Total nitrogen
TOC	Total organic carbon
VPA	Variation partitioning analysis
WEOC	Water extractable organic carbon
θv	Volumetric water content

1. INTRODUCTION

1.1 Objectives

Northern peatlands are increasingly drawing attention due to their important role in the carbon cycle. In peatlands, heterogeneity of water content, nutrient availability, and pH regulate the spatial distribution of biogeochemical processes (Limpens et al., 2006; Vitt, 2006). In addition, the regulating effect of these factors on biogeochemical processes will be affected by a changing climate and lead to positive or negative feedback to atmospheric greenhouse gas concentrations (Yu et al., 2011). In Canada, 12% of the land area is recognized as peatlands, of which over 13000 km^2 are mountain peatlands (Cooper et al., 2012; Zoltai and Vitt, 1995). Mountain peatlands commonly have both underlying and interbedded mineral layers, which may affect the vertical and horizontal movement of groundwater (Chadde et al., 1998). As groundwater is the most important source of nutrients in these mountain peatlands, it is highly likely that effects of sediment on movement of groundwater might influence soil property distributions and influence the biogeochemical processes even further. However, little is known about how these mineral horizons regulate distribution of peat properties, and whether it will indeed further affect the biogeochemical processes. If the presence of mineral horizons does affect the biogeochemical processes, it will be interesting to investigate that how these effects respond to a changing climate. In addition, it is necessary to take functional microbial communities into consideration when studying C and N cycling, as microorganisms are important mediators of biogeochemical cycles (Bardgett et al., 2008).

The goals of this study were to: 1) determine if the mineral horizons affect spatial distributions of soil properties by comparing spatial distribution of key soil properties in soil profiles with and without mineral layers; 2) test whether the mineral layer effect further regulates microbial properties and biogeochemical processes by measuring microbial biomass and communities structure with PLFA and determining potential C and N mineralization rates with incubation methods; and 3) study how mineral horizons influence the response of GHG emissions and concentrations at depth to a changing climate by manipulating temperature and water table in an incubation experiment, identifying the drivers for the changes, biogeochemical processes or microbial community structures.

1.2 Organization of the Dissertation

This dissertation is written in manuscript style. The following chapters are a literature review in Chapter 2 and three studies in Chapter 3 to Chapter 5. As shown in Fig. 1.1, the overall flow of this study began with surveying the complex stratigraphy in a mountain peatland, continued to examining how the complex soil profile affected soil properties distribution and C and N cycling, then concluded with determining how the complex stratigraphy might respond to climate change.

Specifically, Chapter 3 explores how complex stratigraphy affects spatial distribution of key soil properties vertically and horizontally in the mountain peatland and how it further influences N cycling at different depths. From the initial soil survey, three distinct soil types were identified in the peatland: sedge peat/silty mineral/calcareous sediment (PMC) in the southwest zone of the basin; sedge peat/moss peat profiles (PP) in the northeast of the basin; and sedge peat/silty mineral/moss peat (PMP) in the middle. Then, the distribution of key soil properties (θv, TOC, TN and pH) in five layers was mapped for the whole basin. Nitrogen cycling rates were measured at different depths in the three soil types.

Chapter 4 further examines how these soil types affect C mineralization and related peat chemistry, microbial community abundance and structure. Fourier Transform Infrared (FTIR) Spectroscopy was used to study the C functional groups in peat samples. Phospholipid fatty acids (PLFA) was used to describe microbial community structure and abundance.

Chapter 5 extends the research to examine how C and N cycling might change under climate warming conditions. The objectives of this chapter were 1) to study the effect of interactions of peat and mineral sediments on GHG emissions and how they might respond in a changing climate; and 2) to investigate the biogeochemical and microbial reasons of these changes. Based on the results of Chapter 3 and 4, PP and PMC were selected for the microcosm study in this chapter. Peat cores were incubated under temperature-water table treatments and monitored with GHG emissions and concentrations in depths. Then the cores were disassembled to measure biogeochemical processes rates, apparent enzyme activation energy and microbial community. Finally, in Chapter 6, I summarize the major findings of the research and suggest future work.

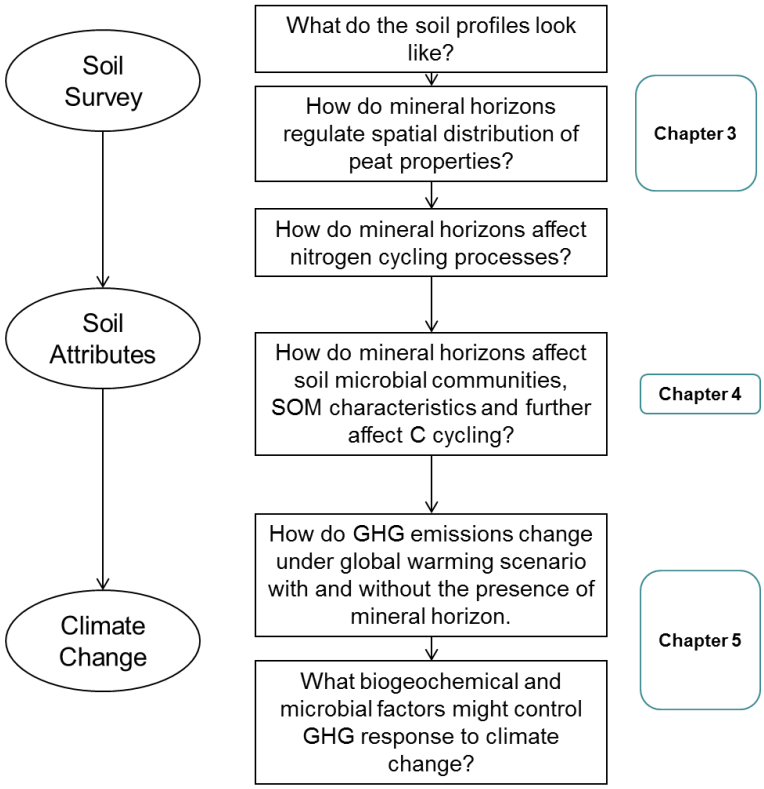

Fig. 1.1. Dissertation organization.

2. LITERATURE REVIEW

2.1 Peatland characteristics and complex peat profiles

2.1.1 Peatland characteristics

"Peatland" is defined as a waterlogged area covered with a naturally accumulated layer of organic substrate of at least 30-40 cm thickness (Glaser, 1987; Joosten, 2008). In Canada, 12% of the land area is recognized as peatlands, among which over 13000 km^2 are mountain peatlands (Cooper et al., 2012; Zoltai and Pollett, 1983). Generally speaking, peat accumulates in wetlands because of oxygen depletion and cool temperatures, which decrease the decomposition rate of organic materials. Considering the large amount of organic carbon, peat is classified in the Canadian System of Soil Classification as Organic soil, although the middle layer of wetland peat is usually dominated by deposited limnic materials (Kroetsch et al., 2011).

Peatlands can be divided into four main types according to their surface and geomorphic development pattern: fens, bogs, swamps and marshes (Tarnocai and Stolbovoy, 2007). Fens, which receive water and nutrients from surrounding groundwater, are usually less acidic and contain more nutrients than bogs, which leads to a growth of grasses and sedges (Joosten, 2008; Kirk, 2004a). Bogs, on the other hand, are usually acidic and oligotrophic with *Sphagnum* spp. moss, because they typically only receive water and nutrients from precipitation (Joosten, 2008; Kirk, 2004a). Swamps and marshes both have standing or slowly flowing, nutrient-rich water. Swamps are usually forested and developed with peat materials from decomposed wood. Marshes, however, are usually developed with decomposed reeds and aquatic materials. According to the nutrient and pH gradients, fens can be further divided into rich fen, transitional fens and poor fens (Vitt et al., 1995). Mountain peatlands are distinct from peatlands formed in other landscapes. High precipitation and cool temperatures make mountain regions ideal for peatlands. Fens, especially rich fens, are usually found in Rocky Mountain regions, because of the dry summer climate (Chadde et al., 1998). These peat bodies are thick (> 4m), which is not typical in other mountain peatlands (Chimner et al., 2010). Mountain fens can be further divided into basin fens and slope fens. The former has more mineral sediments, but is less common than the latter (Cooper et al., 2012).

Nutrient conditions in peatlands are quite different from one to another because of nutrient availability and geomorphological differences. As noted, fens are rich in nutrients and have higher N concentration and lower C/N than bogs (Hugelius and Kuhry, 2009; Limpens et al., 2006). Due to geomorphic differences, alluvial fens in floodplain valleys also have higher organic C and cation exchange capacity (CEC), whereas seepage fens that have slopes intercepting the water table and receive seepage from ground water have higher pH and exchangeable Ca^{2+} (Moorhead et al., 2000). It has also been observed that CEC of fens decreases with increasing depth (Moorhead et al., 2000). Another distinguishing characteristic of peatland soils is the redox condition related to changes of water table. Drier surface soil during summer is expected to increased N mineralization and NO_3^- availability (Niedermeier and Robinson, 2007).

2.1.2 Complex stratigraphy of peat profiles

Peatlands are commonly described as having two layers, acrotelm and catotelm (Bragg and Tallis, 2001), although this model has been recently challenged (Morris et al., 2011). In the two-layer model, acrotelm is the surface active layer that contains living plants like mosses and the growing root system of sedges and has higher hydraulic conductivity. Catotelm is the deeper layer, which consists of non-living and partially degraded plants and has lower hydraulic conductivity (Ingram, 1978). In addition, with the changes of water table and microclimate, peat sub-surface stratigraphy also varies: along the peat profile, the degree of humification changes with depth (Holden, 2009), and peat type usually transits from moss peat to sedge peat (Kuhry and Turunen, 2006). More importantly, alongside with stratigraphy in continuous peat, Morrison (2014) has found complex stratigraphy with presence of mineral sediments in mountain peatlands. In addition, considering multiple environmental factors like paleopond sediments (Morrison, 2014), volcanic ash deposition (Zoltai, 1989), autogenic deposition like marl (Churchill, 1962), floods (Bhiry et al., 2007) and wildfire (Kuhry and Turunen, 2006), the presence of mineral sediments in peatland is expected to be a common feature.

In the Canadian Rocky Mountains, it has been found that beaver activity is very important in peatland formation (Johnston, 2012). Beavers have been modifying wetlands and influencing soil formation since long before humans did by building beaver dams along low-gradient alluvial channels with no rock substrate (McComb et al., 1990). It has been found that beaver have altered around 15% of the land area in certain landscapes; in Alberta 3.23% of the landscape has

been impounded into beaver ponds (Johnston, 2012). The impounded beaver ponds slow down water velocity and therefore contribute to accumulation of sediments (Johnston, 2001). The sediments are a large sink of N and P (Francis et al., 1985; Naiman and Melillo, 1984). It is reported that both NO_3^- and NH_4^+ are higher in beaver ponds sediments than forest soils and stream sediments (Johnston, 2012). This layer of sediment in beaver pond is also a key layer of the soil profile in what is called "beaver meadow". Beaver meadows form from beaver ponds after the dams are abandoned (Terwilliger and Pastor, 1999). Because of the anaerobic conditions in the abandoned pond, decomposition rate is slow which promotes organic matter accumulation on the sediment layer and forms a new layer of organic soil (Rosell et al., 2005). Johnston (2001) described the typical characteristics of a soil profile in a beaver meadow, where below the peat layers (Om and Oh horizons) was a layer of silt (C) covering the previous soil surface (Oh and B horizons). Beaver ponds cause moisture gradients from upland to ponds that can even persist after the ponds are abandoned (Naiman et al., 1994).

Another important source of mineral sediments is volcanic ash. In western Canada, widespread volcanic ash layers in the Holocene were derived from eruptions of Mount Mazama, Mount St. Helen, and Mount Meager within Bridge River tephra region. Mazama ash has been widely identified in Canadian Rocky Mountain areas (King et al., 1982; White and Osborn, 1992; Zoltai, 1989). During the eruption of Mount Mazama around 5677 BC (Zdanowicz et al., 1999) in Oregon, a large amount of fine particle volcanic ash was produced. As volcanic ash is wind spread, with increasing distance from volcano, the grain size become smaller and the thickness of deposited ash layer become thinner (Sarna-Wojcicki et al., 1981). Tephra layers have been found (visible or invisible) in peat profiles throughout Alberta (Oetelaar, 2002; Zoltai, 1989). Atmospheric deposition is not the only way of tephra layer formation. Alluvial depositional of ash layers was identified by Borchardt et al. (1973) in peatlands of eastern Oregon. It has been found that volcanic ash contains more nutrients and electron acceptors, which can promote decomposition and therefore affect peat humification near deposited ash (Broder et al., 2012).

In Northern Rocky Mountain areas, deposits of marl are very common in rich fens (calcareous fens) especially in extremely rich fens (Chadde et al., 1998). Marl is the precipitation of calcium carbonate from calcareous ground water; it is typically fine-grained and found in places where ground water discharges (Miner and Ketterling, 2003). Usually, organic material accumulates on top of this carbonate deposition. In contrast, fens near streams or located in

depositional locations may receive mineral materials from surrounding areas and have more complex stratigraphy. For example, peat profiles may be interbedded with calcareous sediment or sandy and silty sediments (Amon et al., 2002). With higher contents of dissolved minerals, groundwater electrical conductivity (EC) is usually high and pH is close to or higher than neutral (Amon et al., 2002). Due to easier access to nutrient and less acidity, calcareous fens are usually high in botanical diversity and dominated by vascular plants like sedges and willows, but rarely by *Sphagnum* mosses (Amon et al., 2002).

The deposition of mineral sediments, offsite or onsite, creates conditions where mineral sediments and peat geochemically interact. These interactions have been studied from the perspective of mineralogy and groundwater chemistry, suggesting that mineral sediments provide major and trace elements and alkalinity to adjacent peat via groundwater diffusion (Steinmann and Shotyk, 1997a, b). However, little research has been done from the pedological perspective to study the effect of mineral sediments and peat interaction. It has been documented that mineral sediments have lower hydraulic conductivity, which can regulate the vertical and horizontal movement of groundwater (Bragg, 2002; Glaser, 1987). Moreover, when groundwater flows through the mineral sediments, it dissolves nutrients and alkalinity from mineral sediments and moves them to adjacent peat (Charman, 2002). Thus, it is highly likely that mineral sediments not only influence peat profile stratigraphy but also spatial distribution of soil properties in peat profiles.

2.2 Carbon cycling in peatland

The soil C pool is estimated to contain about 2500 Pg C, and is the largest active C pool, estimated to be 3.3 and 4.5 times the size of the atmosphere C pool and the biotic C pool, respectively (Lal, 2004). Among the entire terrestrial ecosystem, peatlands cover the smallest area, about 3% of the total land area (Eglin et al., 2010; Gorham, 1991). However, peatlands not only store about 30% of total global soil C, but also have the highest soil organic carbon (SOC) density, approximately 1140-1430 Mg C ha^{-1} (Eglin et al., 2010; Gorham, 1991). Peatlands in western Canada occupy just 0.255% of the global land area but store 2.1% of global soil carbon (Vitt et al., 2000). Therefore, the carbon storage in peatland soil and carbon exchange between peatland and atmosphere are essential parts of the global C cycle.

Carbon cycling in peatlands is increasingly gaining attention in a changing climate, due to the vast amount of C stored, especially in the northern peatlands, which is reported to be sensitive to global climate change (McGuire et al., 2009). During the Holocene, northern peatlands acted as a sink of CO_2-C and a source of CH_4 under net cooling and warming conditions respectively (Frolking and Roulet, 2007). However, as Moore et al. (1998) stated, the storage of C in peatlands is difficult to predict, because of its sensitivity to environmental controls. Moreover, more studies need to be done to investigate whether global warming leads to positive or negative climate feedback, although more researchers seem to support the latter prediction (Dorrepaal et al., 2009; Yu et al., 2011). Studying the most important characteristics of peatland C, i.e. C accumulation and C related greenhouse gases emission (CO_2 and CH_4) will be helpful in settling questions that remain a challenge.

2.2.1 Carbon accumulation and mineralization

Soil can be both a sink and source of C. Based on the investigation of long-term rates of global soil organic C accumulation, soil C storage increased slowly in the Holocene period (Schlesinger, 1990). It is reported that peatlands serve as a large C sink because of their unique environmental characteristics (Vitt et al., 2000). Peatlands have been accumulating C since the last ice age and continue to increase the total C storage at a rate of approximately 19.4 g m^{-2} $year^{-1}$ (Vitt et al., 2000; Yu et al., 2011). Also, C stored in peatlands roughly amounts to 25-50% of the current atmospheric burden (Frolking and Roulet, 2007). C accumulation in soil represents the balance between net primary production (NPP) and C mineralization (Post and Kwon, 2008). Peatland C accumulation is a result of a greater rate of inputs than outputs. In peatlands, the key processes in C cycling are respiration in the aerobic zone where only seasonal water saturation occurs, and because of the waterlogged condition fermentation, methanogenesis and S, Fe, and nitrate reduction occur in the anaerobic zone (Beer et al., 2008; Kayranli et al., 2010). Aerobic respiration, which only forms CO_2, is far more effective than anaerobic respiration which produces CO_2 and CH_4 via fermentation and methanogenesis (Kayranli et al., 2010). From the anaerobic respiration in deeper peat, organic matter is slowly decomposed and structurally changed *in situ* into recalcitrant carbon compounds. As a result, the highest rates of decomposition are found closest to the surface horizon where the inputs of fresh litter and labile OM are significantly higher (Beer et al., 2008; Schiff et al., 1998).

The changing environmental conditions have great potential to influence C accumulation and mineralization (Schlesinger, 1990). These environmental conditions include soil/air temperature, hydrological condition, nutrient availability, as well as other factors (salinity, acidity, solar radiation) (Craft, 2001). Hydrologic conditions are the most significant characteristic that distinguish peatlands from other ecosystems and help in C accumulation. When oxygen and activities of aerobic microbes are limited, organic C mineralization can only proceed through anaerobic mechanisms, which are not only slow, but also generate toxic products, such as acetaldehyde and ethanol (Reddy and DeLaune, 2008a). Therefore, instead of being quickly decomposed, C tends to be stored under submerged conditions (Moore and Basiliko, 2006). Generally, C mineralization increases with decreasing of water table (Danevčič et al., 2010). It has been found that hydrologic effects on C mineralization also differs by time scale, whereby short term water table changes have a minor impact on C cycling (Deppe et al., 2010). Temperature and soil nutrients are also essential factors that should be taken into consideration when studying C cycling (Craft, 2001). Peak C accumulation rates occurred historically during a warmer climate, as high temperature promoted NPP (Yu et al., 2011). Meanwhile, low temperature restricted decomposition. For example, in northern peatlands, decomposition is quite low in such a cool climate and therefore they are more likely to develop peat (Yu et al., 2009). Soil nutrients, especially N and P, have been demonstrated to influence C cycling (Gorham, 1991). Nitrogen and P are essential to the input of organic material (Oren et al., 2001).

2.2.2 Soil organic matter (SOM) characteristics

In addition to the environmental factors discussed above, another internal factor affecting C cycling is SOC characteristics (Davidson and Janssens, 2006; Schimel and Schaeffer, 2012). In peatlands, SOC quality is the rate limiting factor that controls C cycling by affecting microbial community structure and activity (Schimel and Schaeffer, 2012). Peat materials are exposed to microbes and their only protection is biochemical structure, which is different from mineral soils where C cycling is more controlled by accessibility as SOC are protected by clay and silt (Dungait et al., 2012). One way to assess the lability of SOM is the number of enzymatic steps required to mineralize the organic carbon in the SOM (Bosatta and Ågren, 1999). The more difficult the organic carbon is to be mineralized, the larger the number of steps that is required, and the lower is the quality of SOM (Bosatta and Ågren, 1999). This type of C (recalcitrant C) is

resistant to decomposition by soil microorganisms (e.g. aromatic fractions and other complex and more recalcitrant molecules). In contrast to recalcitrant C, labile C is the major fuel for microorganisms' growth and activity, e.g. cellulose, hemicelluloses, and proteins.

Labile C is active in C and N cycling, whereas recalcitrant C serves as a more stable C pool. Plant residues with high C/N and high lignin/N are classified as low-quality plant material and have been found to decompose more slowly than high-quality plant material (Carvalho et al., 2009; Preston et al., 2006). With increased decomposition, OM composition changes, with increasing recalcitrant C and decreasing labile C (Kalbitz et al., 2003; Updegraff et al., 1995). In peatlands, there is typically more recalcitrant C in subsurface peat and more labile C near the surface (Wright et al., 2011). In addition, according to Arrhenius equation, recalcitrant C is more sensitive to temperature changes, although the changes of absolute rate is likely to be small and difficult to detect (Davidson and Janssens, 2006).

Spectroscopy and water extraction of SOM are both widely used methods in quantifying the C fractions. Water extractable organic matter (WEOM) is the fraction of SOM that can dissolve in water and pass through a 0.45 μm filter (Chantigny et al., 2008). Although both labile and recalcitrant C compounds have been detected in WEOM, WEOM is still recognized as organic substrate that is readily available to soil microorganisms (Chantigny et al., 2008; Murphy et al., 2000). Spectroscopies are considered as less "destructive" methods, focused on biochemical structures (i.e., solid-state ^{13}C nuclear magnetic resonance (NMR) and Fourier Transform Infrared (FTIR)) and physical protection of SOM by mineral particles (i.e., X-ray absorption near-edge structure (XANES)). For FTIR, specific absorption bands are used to describe major fractions of carbon compounds (i.e. polysaccharides, phenolic and aliphatic, aromatic groups, and lignins) (Table 2.1) Broder et al., 2012. This technique has been widely used to measure SOC fractions in peatlands (Artz et al., 2006; Broder et al., 2012; Tfaily et al., 2014) and describe substrates quality via ratios of recalcitrant fractions and labile fractions (Beer et al., 2008).

Table 2.1. Carbon fractions and absorption bands.

Absorption bands	Carbon fractions
950-1170 cm^{-1}	Polysaccharides
~1420 cm^{-1}	Phenolic and aliphatic structures
~1510 cm^{-1}	Aromatic C=C or CO of amide groups
~1630 cm^{-1}	Lignin and other aromatics and aromatic or aliphatic carboxylates

2.3 Nitrogen cycling in peatland

Peatlands in western Canada are a C pool as well as a long term sink for N (Loisel et al., 2014). Nitrogen in peatland ecosystem is mainly from N deposition, biological N fixation and ground water supply. Together with a greater proportion of C stored as peat N is recognized to be limited in northern peatlands. Gorham and Janssens (2005) found that C:N in peatlands in North America range from around 50:1 to more than 100:1 from surface soil to a depth about 3m, which is much higher than other types of soils. Therefore, when SOM decomposition occurs, microbes need to take in N to maintain the relative balance of organisms C:N (i.e. 5:1 to 8:1). Moore et al. (2004) reported an estimated N accumulation rate of 0.2-0.5 g m^{-2} yr^{-1}. Also, it has been estimated that N stored in peatlands is around 8-15 Pg (Loisel et al., 2014). In addition, a modeling study found that under global warming conditions, whether the terrestrial system is a source or sink of C is correlated with C-N interactions (Sokolov et al., 2008). Moreover, the N cycling itself also generates greenhouse gases, N_2O, though aerobic or anaerobic pathways. Emissions of N_2O are reported to be sensitive to temperature, hydrological conditions, etc. and are expected to increase under climate change conditions (Montzka et al., 2011). Therefore, a better understanding of N cycling is needed for accurate estimation of C storage and greenhouse gas emissions in peatlands.

2.3.1 Nitrogen mineralization (ammonification)

Forms of N in peatlands soils are inorganic N like ammonium (NH_4^+) and nitrate (NO_3^-), and organic N like particulate organic N, microbial biomass N and dissolved organic N (DON) (Reddy and DeLaune, 2008b). Organic N is the major form of N in peatlands like in other types of soils, comprising 95% of total soil N. The whole organic N pool can be subdivided into labile N pool (<10% of the total pool size) and recalcitrant N pool (Limpens et al., 2006). The mineralization of organic N mainly takes place in the labile N pool and is more sensitive to

climate change. Nitrogen mineralization (also called ammonification) refers to the breakdown of organic N into NH_4^+. Net N mineralization is the difference of gross N mineralization and N immobilization, that is assimilation of inorganic N by microorganisms into organic N. Both N mineralization and immobilization involve a series of enzymatic reactions and are driven by microorganisms (Reddy and DeLaune, 2008b). It is known to be more accurate to use gross N mineralization to describe the transformation of organic N to ammonium. In peatland ecosystem gross N mineralization rate is 4-10 times of the net rate and is usually positively related to total N (Wray and Bayley, 2008).

As N mineralization is a microbially mediated process, factors that can affect microbial communities are suggested to affect N mineralization. Temperature is one of the most important factors that control microbial activity. Researchers have found in peat incubation experiments that N mineralization increased with rising temperature (Gao et al., 2009; Weedon et al., 2013). Also, net N mineralization in soils of other ecosystems is reported to increase in warming climate (Rustad et al., 2001). However, slower rates of N mineralization were also observed in warmer plots which might result from the interaction of temperature with soil moisture (Groffman et al., 2009), which is suggested to be a more important driver of N mineralization (Yu and Ehrenfeld, 2009). Hydrological conditions that affect oxygen concentration contribute most to the differences of N mineralization in submerged soils. Higher N mineralization was found under low water level but not in fluctuating conditions (Yu and Ehrenfeld, 2009). Other factors, such as C/N and pH, are also controlling factors of N mineralization in peatlands (Chapin et al., 2003; Reddy and DeLaune, 2008b).

2.3.2 Peatland nitrification and denitrification

Nitrification and denitrification are two important steps of N cycling that are responsible for the transformations of different forms of inorganic N that result in N_2O emissions. Nitrification refers to oxidation of NH_4^+ to NO_3^- which is a microbial related two-stage aerobic reaction (Equ. [1]) (Prosser, 2007; Smith et al., 2003). In the first step, NH_4^+ is oxidized into nitrite (NO_2^-) via hydroxylamine (NH_2OH) with O_2 as the electron acceptor. The second stage involves in oxidizing NO_2^- to NO_3^-. The first step is considered to be the rate determining step of nitrification (Sylvia et al., 2005). In addition, this step can also release N_2O into atmosphere, which is referred to as the "hole-in-the-pipe" N_2O production (Davidson et al., 2000; Prosser,

2007). Previously, it was believed that only ammonia-oxidizing bacteria (AOB) could produce N_2O. However, a recent study indicated that ammonia-oxidizing archaea (AOA) are also important in regulating N_2O production; they show no niche differences from AOB with different environmental factors except for pH, as AOA are more dominant in acid soil (Prosser and Nicol, 2012). Denitrification refers to the four reduction processes that reduce NO_3^- to N_2 under anaerobic conditions, which are also microbially regulated (Equ. [2.2]) (Prosser, 2007; Smith et al., 2003). Some denitrifiers (e.g., *A. tumefaciens*) lack the *nosZ* gene and can only produce N_2O as their ultimate product (Wood et al., 2001). In contrast, some *nirS*-type denitrifiers reduce NO_3^- completely to N_2, and hardly produce N_2O (Wittorf et al., 2016). Sometimes, denitrification couples with nitrification, i.e., nitrifier denitrification, which reduces NO_2^- to N_2O and/or N_2, and oxidizes NH_4^+ at the same time (Wrage et al., 2001).

$$\begin{array}{c} \qquad\qquad\quad \text{NO, } N_2O \text{ emission} \\ \qquad\qquad\quad \Uparrow \\ NH_4^+ \Rightarrow NH_2OH \Rightarrow NO_2^- \Rightarrow NO_3^- \end{array} \qquad [2.1]$$

$$\begin{array}{c} \qquad\qquad\quad N_2O \text{ emission} \\ \qquad\qquad\quad \Uparrow \\ NO_3^- \Rightarrow NO_2^- \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2 \end{array} \qquad [2.2]$$

Due to denitrification and microbial uptake, net nitrification is quite low in peatlands, sometime even below zero; therefore, gross nitrification, which can be 500-800 times of net rate in peatland, is now often used to describe the nitrification potential (Wray and Bayley, 2008). Nitrogen cycle is largely mediated by microorganisms; the changes of microbial communities in global warming conditions are suggested to cause changes on nitrification and denitrification potentials (Weedon et al., 2013). Oxygen availability, often related to soil water content is essential factor that controls both nitrification and denitrification rate. Basically, nitrification is inhibited under waterlogged condition and will produce N_2O; however, denitrification requires anaerobic condition and has the greatest rate when 80% of soil pore space is filled with water (Sylvia et al., 2005). Several studies have stated that nitrification rate in peatlands was enhanced by lowering the water table (Chen et al., 2012; Regina et al., 1996). Besides water table, nitrification and denitrification are also impacted by several other factors. Nitrification, as an H^+ release reaction, is often inhibited in low pH conditions (Sylvia et al., 2005). It was observed in an incubation experiment that nitrification rate of the surface layer from drained fen was higher

at pH 6 than pH 4, but in deeper layer nitrification rate was higher at pH 4 because of acetylene inhibition (Lång et al., 1993). Also, the limitation of mineral N inhibits nitrification rate and N deposition is suggested to increase nitrification (Lohila et al., 2010; Regina et al., 1996). Denitrification potential is related with C fraction. Jörgensen and Richter (1992) found that single C components explained 52.1% of the potential denitrification variation. The availability of nitrate is another essential factor that controls denitrification potential. Nitrate generated from nitrification in the surface layer can be leached into deeper layers in the wetland. Therefore, compared with bogs, fens are purported to have higher denitrification rates, because inorganic N is more available in nutrient-rich fens and higher pH in fens does not inhibit nitrification (Limpens et al., 2006).

2.4 Greenhouse gas emissions in peatlands

Biogeochemical processes related to C and N cycling can result in greenhouse gas (GHG) emissions. Considering the large quantity of organic matter stored under water logged conditions, peatland GHG emissions are an essential topic of interest in a changing climate.

2.4.1 Carbon dioxide (CO_2) emission

High C density and C accumulation rates in peatlands contribute to high potential CO_2 emissions. Aerobic (Eq. [2.3]) and anaerobic (Eq. [2.4]) decomposition are the two major pathways of organic carbon mineralization in soil (Reddy and DeLaune, 2008a). In peatlands, mineralization usually follows the anaerobic pathway, which generates less CO_2 but more CH_4 compared with the aerobic pathway.

$$C_6O_6H_{12} + 6O_2 \rightarrow 6H_2O + 6CO_2 \quad [2.3]$$

$$C_6O_6H_{12} \rightarrow 3CH_4 + 3CO_2 \quad [2.4]$$

The ability of peatlands to sequester C is uncertain in a future changing climate, and will largely be influenced by temperature and hydrological conditions (Wunderlich and Borken, 2012). Generally speaking, CO_2 emissions are elevated during warm and dry conditions due to the increase of microbial activity and oxygen concentration in the profile as water levels drop down. In contrast, flooding conditions decreased CO_2 emissions from one northern peatland by 30%-42% (Wunderlich and Borken, 2012). In one field experiment, an greater negative

relationship between CO_2 emission and groundwater table was found with increasing latitude from tropical to boreal regions (Jungkunst and Fiedler, 2007); in boreal regions, CO_2 emissions were more stimulated by lowering the groundwater table. In both field and microcosm studies, it was found that lowering the water table could increase the sensitivity of CO_2 fluxes to increasing temperature, especially for more fibrous peat (Chivers et al., 2009; Kechavarzi et al., 2010).

Peat type is another important factor regulating CO_2 emissions: peat with higher polysaccharides and proteins shows higher CO_2 emissions (Treat et al., 2014). In addition, nutrient-rich peat responded more aggressively to lowering water table compared to nutrient-poor peat (Aerts and Ludwig, 1997). To date, most of the research on CO_2 emissions under global warming focused on the surface layer of peat (Wright et al., 2011). Typically, as depth increases, the availability of oxygen decreases, therefore, the deeper layer under anaerobic conditions should have less CO_2 emission compared with upper layers. Crow and Wieder (2005) found that in northern peatland, 35-57% of the total CO_2 efflux was originated from the peat surface layer. However, in an incubation experiment to imitate global warming conditions, it was found that by increasing the air temperature 1°C , 69% of the CO_2 emission was derived from deeper peat (25-50cm) (Dorrepaal et al., 2009).

2.4.2 Methane (CH_4) emissions

Methane (CH_4) is the second most abundant GHG in the atmosphere with 28-34 times the 100-year global warming potential (GWP) compared with CO_2 (IPCC, 2013). Northern peatlands alone contribute approximately 4~10% of the global CH_4 flux (Mikaloff Fletcher et al., 2004). Methane can be produced by two major pathways: acetotrophy (Eq. [2.5]) and hydrogenotrophy (Eq. [2.6]) (Craft, 2001). Determined by redox condition, CH_4 is largely produced by the first pathway with only a small extent generated from reduction of CO_2 in submerged soil (Kirk, 2004b).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad [2.5]$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad [2.6]$$

Net CH_4 emission involves not only methanogenesis, but also methanotrophy. Usually, CH_4 emissions have a positive feedback relationship to global warming under wetter and warmer conditions (Montzka et al., 2011). Water table levels were found to explain approximately half of

the seasonal variation of CH_4 emissions in Canadian peatlands (Moore et al., 2011). Temperature sensitivity of CH_4 emissions is complex. Most studies conclude that CH_4 emissions are regulated by a combination of hydrological conditions, peat temperature and vegetation (Dinsmore et al., 2009; Moore et al., 2011). In addition, similar to CO_2, the positive correlation of CH_4 emissions to water table levels also exhibits latitude related relationships; in higher latitude locations, CH_4 emissions increase more intensively as water table levels increase (Jungkunst and Fiedler, 2007). Moreover, although it is not documented as well as other factors, the quality of substrate such as carbon compound quality, is also an important regulation of CH_4 emissions (Wright et al., 2011). In incubation studies, the effect of temperature and water table on CH_4 emissions is also well documented. It has been found that lowering water table after stopping irrigation decreased CH_4 emissions, which was more reflected in bog (Deppe et al., 2010). This is because sedges in fens accelerated soil air transportation by capillary fringe and resulted in higher CH_4 emissions in fens (Deppe et al., 2010). Higher temperature increases CH_4 production, not only because it stimulates microbial enzyme activity, but also because it shifts the CH_4 production pathway (Chin and Conrad, 1995). In addition, similar to CO_2 emissions, CH_4 emissions are correlated with substrate quality: substrates with a greater degree of decomposition are likely to have lower CH_4 emission (Reiche et al., 2010), and materials with lower C quality are expected to be more sensitive to increasing temperature (Inglett et al., 2012). Last but not least, despite all the above regulating factors, CH_4 production rates are highly spatially variable, even in microcosm experiments (Reiche et al., 2010).

2.4.3 Nitrous oxide (N_2O) emission in peatland

Nitrous oxide (N_2O) with total global emission of 18.8 Tg N_2O-N $year^{-1}$, is the third most abundant GHG in the atmosphere and it is equivalent to approximately 298 times global warming potential (GWP) of CO_2 (IPCC, 2007). Lower N_2O emissions from peatlands, compared with CH_4 and CO_2, is due to low mineral N availability (Marushchak et al., 2011). However, high N_2O production potential is expected to occur under global warming (Elberling et al., 2010). Nitrous oxide is generated by two major processes: nitrification and denitrification. The optimum hydrological condition for N_2O emissions is at 60-80% WFPS where oxygen concentrations are not too low so that denitrification does not fully proceed to N_2 and rather, produces N_2O (Davidson et al., 2000).

In northern peatlands, researchers have found that N_2O emission would increase under drier conditions even during short-term drainage, because the lower water table is able to stimulate N mineralization that can supply more inorganic N (Martikainen et al., 1993; Regina et al., 1996). Meanwhile, fluctuating hydrological conditions are reported to generate the highest N_2O emissions (Davidson et al., 2000). In incubation studies, it has been found that N_2O achieved peak flux rates when the water table was around 20 cm below surface (Jungkunst et al., 2008). Beside oxygen availability, several studies have stated that C availability had a substantial contribution to N_2O fluxes, as C availability stimulates microbial activity and therefore increases N_2O fluxes (Danevčič et al., 2010). With higher content of available C, more N_2O was likely to be lost into the atmosphere when temperature increased (Wang et al., 2014). In addition, N availability and other nutrients are also positively correlated with N_2O fluxes: minerotrophic fens usually have higher N_2O emissions than ombrotrophic bogs, which are sometimes even lower than detection limit (Regina et al., 1996). Again, N_2O fluxes are highly spatially and temporally variable at large or small scales (Giles et al., 2012).

2.5 Microbial communities in peatlands

Microorganisms mediate the key steps of C and N cycling (Prosser, 2007). With the increase of microbial activity directly caused by warming conditions, biogeochemical processes are more likely to change and result in positive or negative feedback to climate change. Therefore, it is important to understand the mechanism of microbial changes under global warming conditions.

2.5.1 Microbial community structures and abundance in peatland soils

Microbes in peatland ecosystems are known to be enormous in population and variety (Andersen et al., 2013). Within peatlands, it has been observed that microbial community structures are different along nutrient gradients, with depth, as well as between different seasons (Jaatinen et al., 2007). Firstly, along the soil profile, similar to agricultural soils, microbial biomass in peatlands decreases with increasing depth (Helgason et al., 2014; Preston et al., 2012). This results from the combined effect of decreasing temperature and oxygen availability (Andersen et al., 2013) and increasing recalcitrant C and depletion of labile C in deeper layers (Basiliko et al., 2007). In addition, with depth, not only does microbial abundance decrease, but the microbial communities change. Oxygen availability is an important regulator: fungi are less

adapted to anaerobic environment conditions, so bacteria to fungi (B:F) biomass ratios increase with depth and bacteria is recognized as the dominant decomposer in deeper anaerobic layers (Killham and Prosser, 2007). As C in deeper peat is older compared to C in surface layer, the biomass of Gram positive bacteria (Gram+) that is more likely to use recalcitrant C increases with depth; meanwhile, the biomass of Gram negative bacteria (Gram-), that is more likely to use labile C, decreases with depth (Jaatinen et al., 2007). In addition, bacterial communities are quite different from each other at different depths of peat profile; however, no relationships have been found between structural diversity of the bacterial community and peat depth (Kim et al., 2012). As for microbial activity changes with depth, it was observed that anoxic substrate induced respiration (SIR) ratios and enzyme activity decreased with depth; however, oxic SIR ratios increased with depth (Preston et al., 2012).

Secondly, microbial community activity, abundance and structures differ from ombrotrophic bogs to minerotrophic fens due to nutrient, pH and other chemistry gradients (Andersen et al., 2013). In general, higher microbial activity and abundance have been observed in fens compared to bogs, as fens are more favorable for microbes with neutral pH, and more nutrient and electron acceptors (Moore and Basiliko, 2006). Specifically, for nutrient impact, across different peatland types, fungi are the dominant microbial group in ombrotrophic bogs, as fungi are more capable of decomposing nutrient poor litter than bacteria (Winsborough and Basiliko, 2010). Acidity is another factor significantly affecting microbial community structure. For example, Bacteria: Fungi ratio decreased with pH as fungi are more adapted to acidic environments (Thormann et al., 2004). Therefore across peatland types, bacteria are more abundant in minerotrophic fens, whereas fungi is are the more abundant in ombrotrophic bogs (Golovchenko et al., 2007). In addition higher bacterial diversity is usually found in neutral pH environments, like fens (Rousk et al., 2009). However, when the variation of pH is not large, pH is not very important in regulating microbial community structure or diversities (Preston et al., 2012).

2.5.2 Response of microbial community structure to climate warming

It is believed that microbial communities are not stable under global warming scenario. Higher temperature tends to stimulate microbial activity and growth. However, microbial biomass could decrease with increased temperature, if microbes suffer from starvation due to the

depletion of labile C (Wei et al., 2014), or if microbial C use efficiency decreases under high temperature as more C is decomposed into CO_2 (Wei et al., 2014). Microbial community structures may also change under increasing temperature, as individual microbial species could respond differently to increasing temperature (Wei et al., 2014). Laboratory incubation experiments found that increasing temperature increased Gram+ abundance but decreased Gram- and fungi abundance (Biasi et al., 2005). In the field, temperature changes only impacted microbial communities in upper layers, whereas little impact was found on deeper layers (Kim et al., 2012). In addition, hydrological conditions and vegetation patterns changed under climate warming condition (Roulet et al., 1992), which also contributed to the changes of microbial community structure in peatland ecosystems (Jaatinen et al., 2007). Moreover, changes of microbial community structure are not consistent in fens or bogs. As noted by Jaatinen et al. (2007), fungal biomass increased in mesotrophic fen, but decreased in ombrotrophic bog under water-level drawdown condition.

Microbial activity is expected to respond more readily to environmental changes than microbial biomass and community composition. Temperature dependence of microbial community activity can also be reflected by apparent enzyme activation energy (E_a) (Yavitt et al., 2000). Basically, higher E_a indicates more energy is required to process a reaction (Davidson and Janssens, 2006), and more sensitivity to temperature changes according to Eq. [2.7], which shows the linear relationship of ln(k) to 1/T (Holtan-Hartwig et al., 2002).

$$\ln(k) = \frac{-E_a}{R}\frac{1}{T} + \ln(A) \qquad [2.7]$$

Where k is the process rate (mol kg^{-1} s^{-1}), T is the Kelvin temperature (K), R is the gas constant (0.008314 kJ K^{-1} mol^{-1}), and A is the Arrhenius constant (mol kg^{-1} s^{-1}).

The E_a of a reaction is regulated by substrate quality and microbial community structure: higher E_a usually indicates more recalcitrant material, as discussed in session 2.2.2; different microbial community structures produce different extracellular enzymes, which results in different E_a (Davidson and Janssens, 2006; Sinsabaugh, 1994). It has been found E_a of GHG-related reactions change with changes of temperature, hydrology interactions and sediment types (Inglett et al., 2012; Lloyd and Taylor, 1994; Yavitt et al., 2000). The E_a of methane production was found to be higher in peat or humic sediments (C:N ratio >10) than that in mineral sediments

(Duc et al., 2010). Higher E_a (Q_{10}) of CH_4 production was found from cold areas than from warm areas (Lloyd and Taylor, 1994). Although there were no significant differences of E_a (Q_{10}) for CH_4 and CO_2 observed in different depth, increased E_a (Q_{10}) value have been reported from subsurface peat when considering temperature and hydrology interactions (Yavitt et al., 2000). Temperature dependence of N_2O production and consumption, as well as its interaction with substrate quality or depth, original temperature and/or water condition are not well studied. In peatland microcosm, N_2O emission increased with increasing temperature (Dinsmore et al., 2009). Therefore, it is expected that E_a of N_2O production is higher than that of N_2O consumption.

2.6 Conclusion

Northern peatlands store a great amount of C (approximately 30% of global SOC) and are very important in C and N cycling. Peat stratigraphy in mountain peatlands is very complex, interrupted with mineral sediments. However, few studies have focused on this complex stratigraphy and little is known about whether it will affect soil properties and C and N cycling. In addition, it remains unknown whether the complex soil profile will affect the response of C and N cycling to a changing climate. This project was conducted to study the complex peat profile in mountain peatlands from pedological perspectives. Specifically, it focused on studying the effect of mineral sediments on spatial distribution of soil properties and N cycling, investigating the influence of mineral sediments on C cycling and associated impact factors like substrate quality and microbial communities, and then testing whether the complex soil profiles affect GHG emission under climate warming condition, including the changes of related microbial and biogeochemical properties.

3. Effect of Mineral Horizons on Spatial Distribution of Soil Properties and N Cycling in A Mountain Peatland[1]

3.1 Preface

Northern peatlands are known for their capacity of accumulating organic matter. Large amounts of carbon (C) and nitrogen (N) are stored as organic forms in peatland soils. Therefore, northern peatlands play an important role in global C and N cycling, and their feedback to climate warming remains unclear. In Canada, 12% of the land area is recognized as peatlands among which over 13000 km^2 are mountain peatlands. In geomorphically unstable mountain areas, peat profiles are usually developed with shallow underlying and interrupted mineral sediments. Mineral sediments have been found to affect groundwater hydraulic conductivity and groundwater chemistry, which are related to soil properties. However, few researchers have studied mineral sediments in peat profile from a pedological perspective. Thus, it remains under discussion whether and how the mineral sediments will affect soil properties and C&N cycling in the stratigraphically complex peatlands. The goal of this chapter is to provide a first investigation and to figure out whether the stratified mineral horizons affect the spatial distribution of soil properties and N cycling rates.

[1] Wang, X., C. Westbrook and A. Bedard-Haughn. 2016. Effect of mineral horizons on spatial distribution of soil properties and N cycling in a mountain peatland. Geoderma 273: 73-82. Xiaoyue Wang is the major contributor and author of the manuscript. Cherie Westbrook is the committee member and helped with experimental design, sampling and writing. Angela Bedard-Haughn is the supervisor and helped throughout this study.

3.2 Abstract

Soil profiles in mountain peatlands commonly show complex stratigraphy with both underlying and interbedded mineral sediments. The presence and types of mineral sediments may affect nutrient gradients via the vertical and horizontal movement of groundwater, which further influences biogeochemical processes, especially for deeper peat adjacent to mineral sediments. To study influences of stratified mineral sediments, we conducted this study in a mountain peatland with three different soil types: sedge peat/silty sediments/calcareous sediments (PMC), sedge peat/silty sediments/moss peat (PMP) and sedge peat/moss peat (PP). Our results indicate that spatial distributions of TOC, TN, pH and θv in this mountain peatland are regulated by mineral sediments. The influence of mineral sediments was more significant in deeper layers. Calcareous sediments strongly influenced spatial distribution of pH. Silty sediment layers influenced θv via slowing water infiltration into deeper layers. The pH and θv affected organic matter decomposition and thus TOC and TN distribution. At the same time, mineral sediments – especially the presence of calcareous sediments – also affected N cycling processes: net nitrification in PMC was higher than that in PP and PMP whereas net ammonification in PMC was lower. As expected, immobilization and nitrification decreased with depth. In addition, with depth, a lag phase for N cycling was found in deeper layers: immobilization and nitrification did not occur until after 7 days in middle peat or 28 days in deep peat in surface-like conditions. Moreover, the interaction of incubation time and soil types showed that the lag phase was different in different soil types. Overall, results suggest that interbedded mineral sediments in mountain peatlands can influence soil hydrology and pH, which in turn affect spatial distribution of soil properties and associated biogeochemical processes.

3.3 Introduction

Peatlands are an important global resource owing to their large C and N storage capacity, due to waterlogged condition (Loisel et al., 2014). Nutrient pool size and rate of cycling are influenced by peat composition, soil hydraulic properties and temperature (Bragg, 2002; Bridgham et al., 1998; Holden, 2009). These insights have been gained mainly from studying peatlands with continuous peat profiles. The peat archive though is revealing that peatland stratigraphy can be considerably more complex (Margalef et al., 2013), particularly in

geomorphically unstable mountain environments (Sewall et al., 2015). There exists only a small body of research on mountain peatlands, but emerging from this literature is that peat deposits can range from thick to very thin, can greatly vary in peat type and organic matter content (Charman et al., 1995; Morrison et al., 2015), or have a profile wherein there are frequent interruptions by mineral or ash lenses (Chadde et al., 1998; Kubiw et al., 1989; Morrison et al., 2015). Little is known about how such stratigraphic complexity affects peatland pedological properties and biogeochemical function.

Mineral sediments have higher bulk density and lower porosity than peat and therefore have lower hydraulic conductivity than peat (Hunt et al., 1996), which is expected to regulate the vertical and horizontal movement of groundwater. Groundwater movement velocity and soil moisture affect organic matter accumulation and decomposition via effects on acidity and reducing conditions (Chow et al., 2006; Morris and Waddington, 2011). Moreover, weathering processes in mineral sediments can release major and trace ions and carbonate. These solutes can migrate upward into the peat profile via upward groundwater flow and diffusion and change groundwater chemistry (Steinmann and Shotyk, 1997a, b). Groundwater chemistry, especially alkalinity and nutrient content, is associated with peat accumulation and decomposition (Beer et al., 2008; Blodau, 2002; Curtin et al., 1998). Given that mineral sediments change groundwater chemistry and are likely to regulate groundwater movement, we hypothesize that mineral sediments influence peat profile development and horizontal and vertical distribution of soil properties. In addition, compared with surface horizons, deeper horizons are expected to be more influenced by mineral sediments due to greater groundwater influence.

The presence and types of mineral horizon may not only influence the distribution of peat properties, but also some biogeochemical processes, like N turnover (Chapin et al., 2003). The large quantities of N (8-15 Pg) stored in peatlands is mostly in the organic form (Gorham, 1991; Limpens et al., 2006). Across peatland types, N cycling rates have been well studied: the accumulation and mobilization of different N forms is controlled by fluctuations in water tables, nutrient availability, substrate quality and pH (Bayley et al., 2005; Bridgham et al., 1998; Chapin et al., 2003; Macrae et al., 2013; Mettrop et al., 2014). However, there hasn't been much investigation into how N cycling in the peat profile is impacted by interbedded and underlying minerals. Based on current knowledge, it is hypothesized that the presence of stratified mineral

horizons affect N cycling rates in peatlands, especially where the mineral sediment has a markedly different pH than the peat matrix (Aciego Pietri and Brookes, 2008).

To test our hypotheses, we compared peat profiles from a mountain peatland with and without mineral horizons to: 1) determine if the presence and types of stratified mineral horizons affect the spatial distribution of soil properties (i.e., total OC, total N, pH, and θv); 2) evaluate whether deeper peat properties are more affected by mineral sediments than upper peat; and 3) quantify N cycling rates as related to presence of stratified mineral horizons.

3.4 Material and Methods

3.4.1 Sites description

This study was conducted in the Sibbald research wetland in the Kananaskis region of southern Alberta, Canada (51.06 N, 114.87 W) (Fig. 3.1A). The selected peatland for this study is a rich fen with hummocky microtopography located in the foothills of the Canadian Rocky Mountains within a relatively level valley at 1480 m a.s.l. (Janzen and Westbrook, 2011). Mean air temperature is -6.7°C for January and 14.5°C for July and mean annual precipitation is 653 mm, as recorded by University of Calgary Biogeoscience Institute (17 km west of the research area; Janzen and Westbrook, 2011). The peatland receives surface water from four creeks that originate from groundwater springs. Two of these creeks converge in the northern extent of the peatland, and form Bateman Creek, a 1-m wide channel that drains the peatland (Fig. 3.1B). The other two creeks (East Inlet and West Inlet) are channelized on the hillslopes, but their flow disappears completely below the ground surface at the peatland margin. East Inlet showed the highest EC, pH and ion concentration followed by downstream and then upstream reaches of Bateman Creek (Table 3.1). There is beaver activity throughout the peatland, and two large beaver ponds are present in the north and southeast areas. The study area is located in the southern half of the peatland.

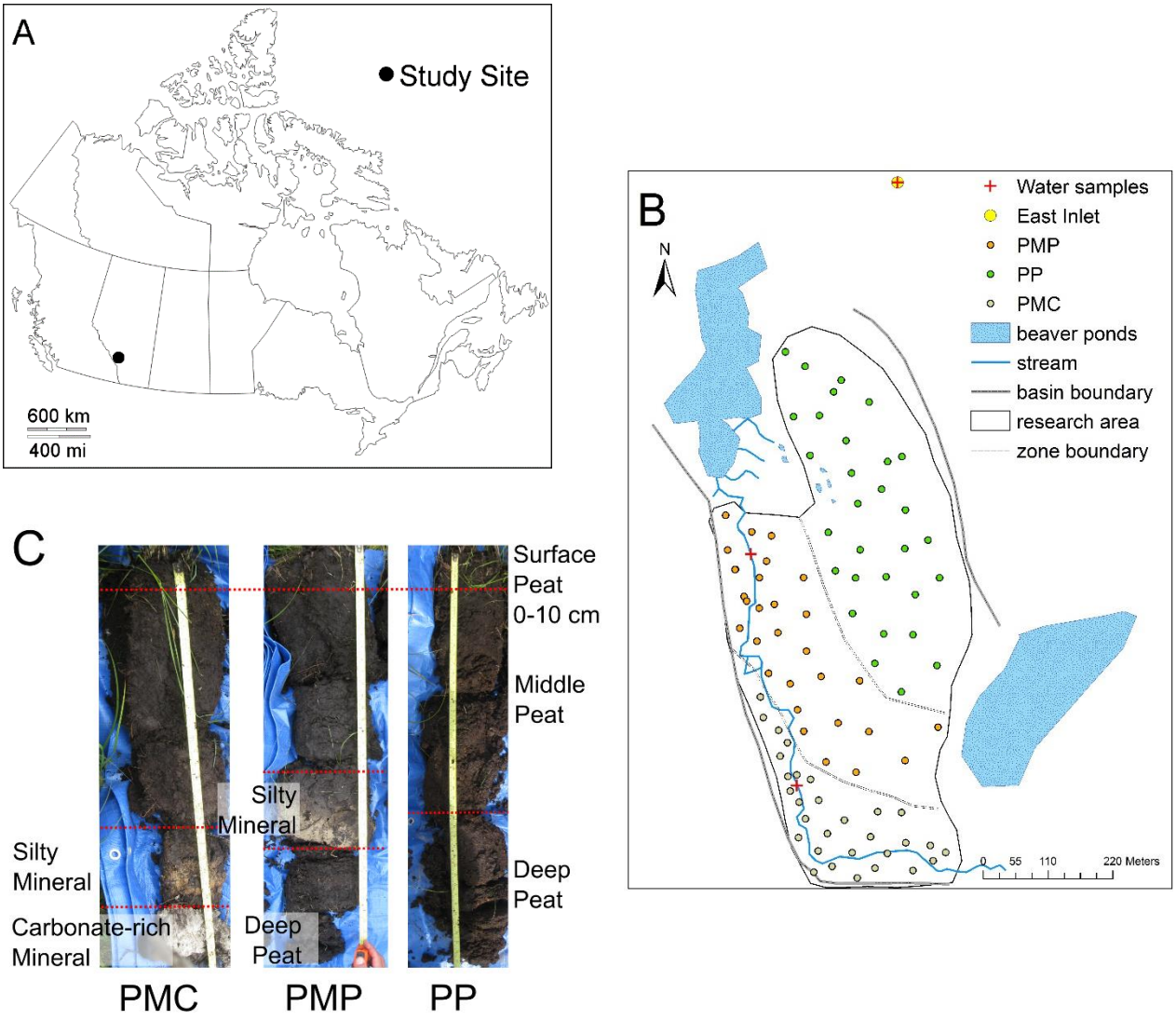

Fig. 3.1. A) Location of the field site. B) Sampling points and soil zones. C) Profiles description from each zone, left to right: PMC (peat/silty mineral sediments/calcareous sediments), PMP (peat/ silty mineral sediments /peat) and PP (sedge peat/moss peat).

Table 3.1. Creek water chemistry at East Inlet and Bateman Creek (upstream reach and downstream reach).

	East Inlet	Upstream of Bateman Creek	Downstream of Bateman Creek
	mg L^{-1}	mg L^{-1}	mg L^{-1}
HCO_3^-	198.00	161.00	217.00
CO_3^{2-}	0.00	0.00	19.00
Cl^-	0.00	0.00	1.00
OH^-	0.00	0.00	0.00
PO_4^{3-}	0.00	0.00	0.00
NO_3^-	0.09	0.04	0.09
NH_4^+	0.38	0.43	0.55
Ca^{2+}	34.00	33.00	41.00
Mg^{2+}	12.00	11.00	16.00
K^+	0.60	0.20	0.20
Na^+	8.00	3.80	5.20
SO_4^{2-}	6.80	1.50	2.20
EC†	301.00	236.00	362.00
pH	8.06	8.15	8.66

†EC: Electronic conductivity (µS cm^{-1}).

A preliminary soil survey found that surface peat originates from sedges (*Carex* spp.) with 40-50% fiber content; deeper peat originates from *Sphagnum* spp. moss, which is less decomposed (60% fiber content). The changes of dominant vegetation indicate a historical shift in climate, as sedges prefer drier conditions (Gunnarsson et al., 2002). Also, in the west half of the research area, a ~0.28 m thick mineral horizon of silty-loam occurs at a depth of ~0.65 m below surface (Table A.1). In the very south end, a horizon of calcareous sediment (average thickness 0.48 m) was identified at an average depth of 1 m (Table A.1). Although these calcareous sediments underlie a large portion of the peatland in the north part, they are at depths greater than 4-5 m, whereas we restricted our investigation to the surface 0-150 cm. Therefore, three different types of organic soils were delineated according to the arrangement of mineral and organic horizons (Fig. 3.1B). In the southwest zone of the basin, the soil profiles consisted of a layer of sedge peat, underlain by a thin silty mineral deposit lying directly over calcareous sediment (PMC); in the middle zone, the soil profile has a layer of sedge peat, interbedded with a thin silty mineral deposit, lying on top of moss peat (PMP); in the northeast zone, the soil profile

is sedge peat accumulated over more than 4 m of moss peat (PP) (Fig. 3.1C and Table 3.2). In PMC and the west part of PMP, sedges are the most common plant type. In PP and the east part of PMP, sedges and willows (*Salix* spp.) co-dominate.

Table 3.2. Generalized description of horizons in three soil types.

Soil classification†	**Canadian Soil Classification**	**Limnic Humisol**	**Cumulic Humisol/Mesisol**	**Typic Mesisol**
	Profile description	**Peat/silty mineral sediments/calcareous sediments (PMC)**	**Peat/ silty mineral sediments /peat (PMP)**	**Sedge peat/moss peat (PP)**
Soil profile description (depth to surface, cm)	0-10	Carex sedge mean thickness: 47.5 cm range: 26-76 cm	Carex sedge mean thickness: 63.9 cm range: 30-72 cm	Carex sedge mean thickness: 41.4 cm range: 28-70 cm
	10-20			
	20-30			
	30-40			
	40-50			*Sphagnum moss* mean thickness: NA range: 108+ cm
	50-60	Silty loam mean thickness: 25.6 cm range: 10-55 cm		
	60-70		Silty loam mean thickness: 28.3 cm range: 7-52 cm	
	70-80			
	80-90	Calcareous sediments mean thickness: 48.2 cm range: 12-78+ cm	*Sphagnum* moss mean thickness: 32.2 cm range: 7-68+ cm	
	90-100			
	100-110			
	110-120			
	120-130+			

† Soil classification: all three soil types were Histosols in the World Reference Base in Soil Resources (WRB).

3.4.2 Spatial distribution of soil properties

Thirty sampling points were randomly selected from each of the three soil types (Fig. 3.1B). At each point, soil samples were taken to an average depth of 1.3 m; this sampling depth permitted equal thickness above and below the silty mineral sediments in the PMP and included the calcareous sediments in the bottom of the PMC. The GPS coordinates were recorded for each point, and the thickness and pedologic characteristics of each key horizon were described in-field. In the lab, cores were subdivided into five layers according to stratified mineral horizons and depth (Fig. 3.1C): Surface layer referred to the top 10 cm, which is most biologically active due to high concentrations of roots and microbes (Dedysh et al., 2006; Liu et al., 2012; Preston et al., 2012); for mineral sediments, silty mineral horizons and calcareous mineral horizons were sampled as distinct individual layers; for the rest of the profile, peat samples were collected above and below the silty mineral horizons where there were changes of fiber content and von Post test. If there were no differences of fiber content or von Post test, but a given horizon was thicker than 40 cm, it was still separated into two layers. Three representative pits from each of the PMC and PP and one representative pit from PMP were excavated for bulk density measurement.

Gravimetric water content (θg) was calculated by measuring the weight loss of 5 g soil samples after they were dried at 70°C for peat (Russell and Voroney, 1998) and 105°C for mineral soil. Volumetric water content (θv) was calculated from θg and bulk density. Total organic carbon (TOC) was measured by dry combustion using a carbon analyzer (model Leco-2000, Leco Corporation, St. Joseph, MI), after removing carbonates by HCl acid fumigation in a desiccator (Bisutti et al., 2004). Total nitrogen (TN) was also measured by dry combustion with a CNS analyzer (model C632, Leco Corporation, St. Joseph, MI) (Rutherford et al., 2008). Soil pH was determined in 20 mL 0.01 *M* $CaCl_2$ with air-dry samples (10 g mineral soil or 2 g peat samples) by digital pH meter (PC700 pH/mV/cond, Oakton, Vernon Hills, USA) (Hendershot et al., 2008).

The spatial distribution of soil properties in each horizon was mapped in the whole basin by universal kriging in ArcGIS (ESRI, Redlands, California, USA). The distribution of soil properties in different depths and soil types were described by nonmetric multidimensional scaling (NMDS) using the vegan package in R ver. 3.1.2 (R Development Core Team, 2014).

The range and spatial dependence (SPD) were calculated in each layer from each zone by semivariance via GS+ ver. 9.0 (Gamma Design Software, Plainwell, Michigan, USA). The rate of spatial dependency was determined according to Stelzenmüller et al. (2005).

3.4.3 Nitrogen cycling

Based on the semivariance results, eight representative independent sampling points were selected from each of the three soil types. The different depths of peat layers described above were also included in this study. In order to compare means among soil types and depth classes more conveniently and according to peat properties and observation in field, layers 1 to 5 were grouped into three depth classes: surface peat, middle peat and deep peat. Specifically, layer 1 in all three soil types was called surface peat; layer 2 or layer 2 and 3 (peat layers above mineral sediments in PMC and PMP, or sedge peat in PP) were grouped as middle peat; layer 4 and 5 or layer 5 (moss peat below mineral sediments in PMP, or moss peat in PP) were grouped as deep peat.

To quantify ammonification and nitrification, four subsamples (15±0.5 g field moist weight) from each sampled point were put into four vials and incubated at surface-like condition (22°C at field moisture, listed in Table A.3) for 63 d (Izaurralde et al., 2004). Gravimetric water content was monitored every other day and distilled water was added, if needed, to maintain field-moist conditions. One subsample was extracted immediately to provide initial NH_4^+-N and NO_3^--N concentrations: 5 g (5±0.02 g) soil was extracted with 50 mL 2 M KCl and analyzed for NH_4^+-N and NO_3^--N by autoanalyzer. At 7, 28 and 63 d, one subsample vial was extracted for final NH_4^+-N and NO_3^--N concentration. Pool size changes of NH_4^+-N and NO_3^--N were monitored. Net ammonification rate (R_{amm}) and net nitrification rate (R_{nit}) during 0-7, 7-28 and 28-63 days were calculated by the difference in NH_4^+-N and NO_3^--N between corresponding final and initial readings. Rates during 0 to 28 days were selected to discuss the effects of soil types and depth classes, because incubating soils for short or too long of a period can both cause bias in estimating net N mineralization: if too long, the depletion of NH_4^+-N, nitrification might be underestimated (Drury et al., 2008) and if too short (less than 14 days), soil with high C:N ratio can immobilize available N (Curtin and Campbell, 2008).

Homogeneity of variances was tested by Bartlett test and normality was assessed using the Shapiro-Wilk test and histograms. To transform data to normal distribution, nitrification rates

over 28 d were transformed by cubic root; for daily N cycling during day 0-7, day 7-28, and day 28-63, nitrification and mineralization rates were transformed by square root of five, and ammonification rate were transformed by cubic root. Analysis of variance (ANOVA) was used to determine differences in N cycling rates among three soil types (PMC, PMP and PP) and also among three peat depth classes (surface peat, middle peat and deep peat) by fitting a general linear model using the glm function in R ver. 3.1.2 (R Development Core Team, 2014). Correlations between soil properties including SOM characteristic parameters and potential biogeochemical process rates were examined using the Pearson correlation in R ver. 3.1.2 (R Development Core Team, 2014).

3.5 Results

3.5.1 Spatial distribution of soil properties and spatial dependence

Spatial distribution of water content in this peatland was driven by two factors: proximity to the creek and mineral sediments. First, in layers 1 and 2, θv was lower in locations near the creek (Fig. 3.2A & 3.2B); drainage from this peatland via the creek lowered the water table in the riparian area. Second, in deeper layers, θv distribution in soil profiles with vs. without mineral horizons showed distinct patterns. Above the mineral horizon (Fig. 3.2C), peat in soil profiles with mineral horizons had higher θv (PMP: 1.12 ± 0.42 cm^3 cm^{-3} and PMC: 1.00 ± 0.40 cm^3 cm^{-3}) than that in PP (0.73 ± 0.15 cm^3 cm^{-3}); however, below the mineral horizon (Fig. 3.2E), peat in PMP had lower θv (0.52 ± 0.17 cm^3 cm^{-3}) than that in PP (0.79 ± 0.23 cm^3 cm^{-3}). When a mineral horizon was present, more water was stored in the middle peat layer above it.

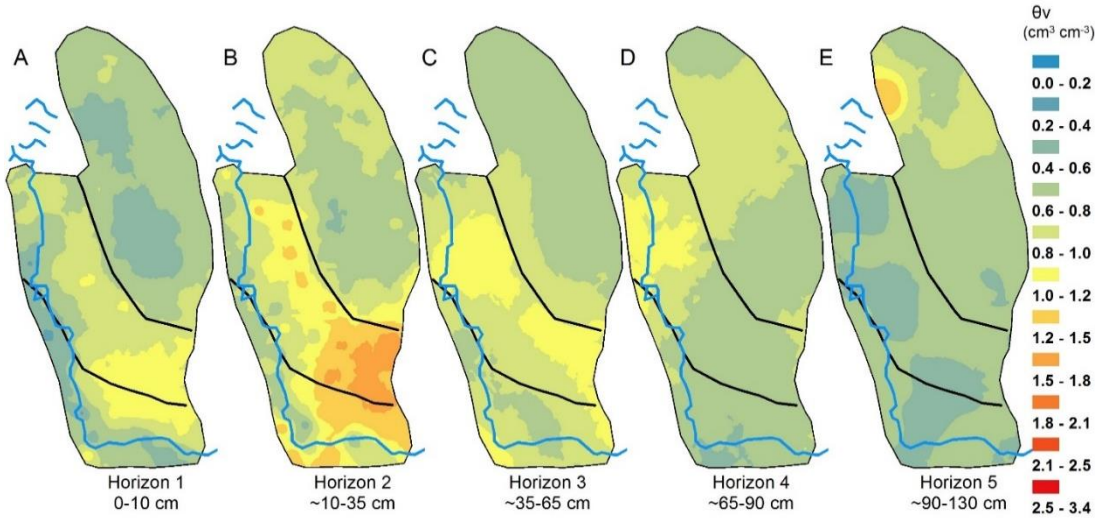

Fig. 3.2. Spatial distribution of θv in each of the five horizons; average depths of upper and lower boundaries are also shown for each horizon.

Total organic carbon and TN varied mostly by parent material: TOC and TN in peat were about eight and seven times greater than in mineral sediments, respectively. In layers 1 and 2, TOC and TN content were lower near the creek (Fig. 3.3A, 3.3B, 3.4A and 3.4B). The effects of mineral materials are greatest in deeper layers. In deep peat (Fig. 3E), lower TOC was found in PMP (38.47±6.10%) compared with PP (45.72±4.24%). Again, compared with PP (2.70±0.39%), lower TN content (2.23±0.62%) was found in PMP (Fig. 3.4E). In the top two layers, both TOC and TN distributions were very homogeneous (Fig. 3.3A, 3.3B, 3.4A and 3.4B), except the changes caused by creek. However, in lower layers, affected by mineral layers, TOC and TN was more heterogeneous (Fig. 3.3C-E, 3.4C-E).

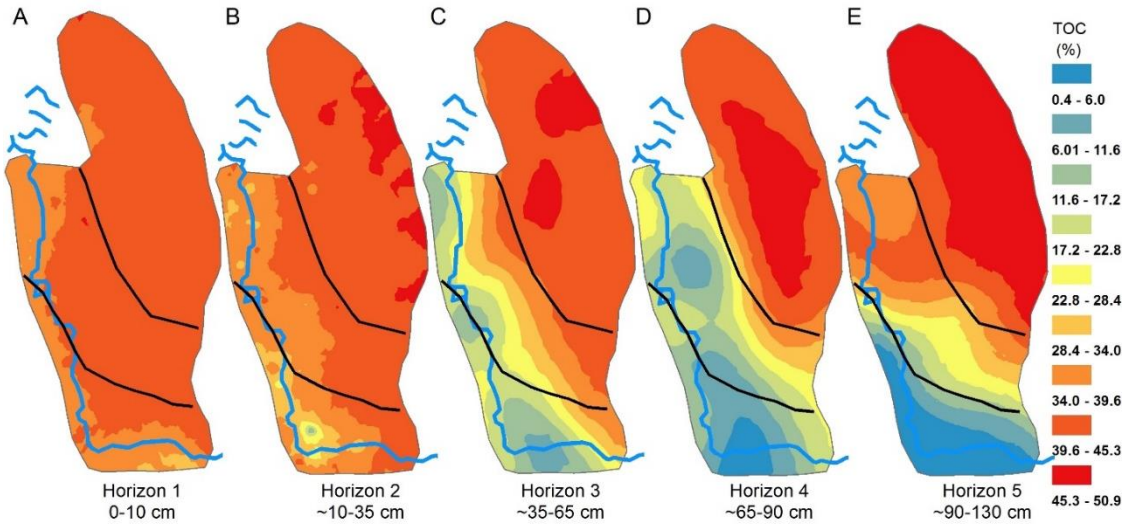

Fig. 3.3. Spatial distribution of TOC in each of the five horizons; average depths of upper and lower boundaries are also shown for each horizon.

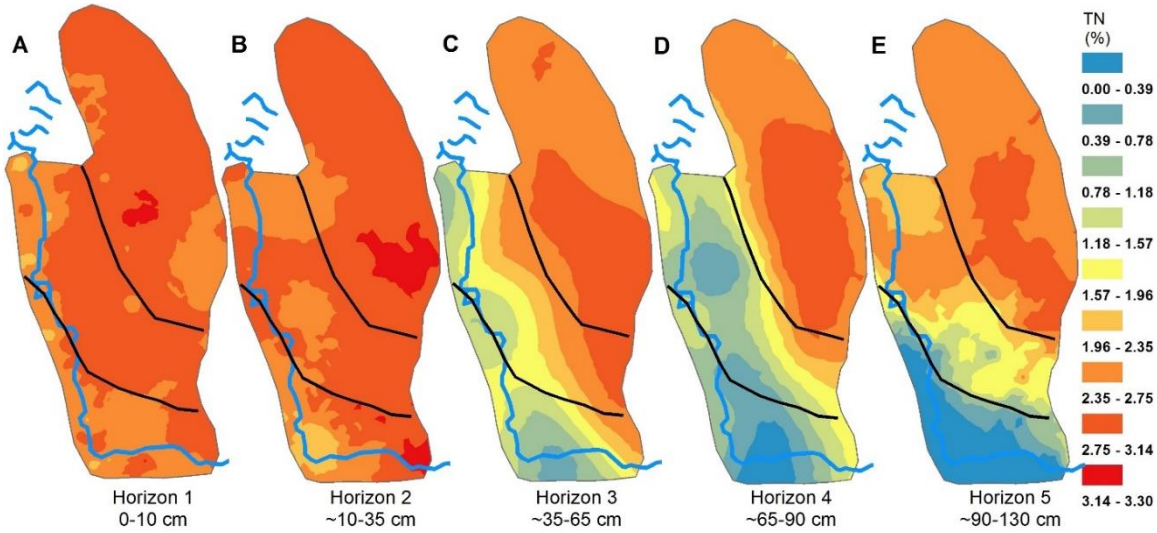

Fig. 3.4. Spatial distribution of TN in each of the five horizons; average depths of upper and lower boundaries are also shown for each horizon.

There was a clear spatial pattern of pH both vertically and horizontally (Fig. 3.5). Vertically, pH in PP decreased with increasing depth (5.79±0.48, 5.52±0.21, 5.42±0.18 in layer 1, 3, 5 respectively, F=10.96, *p*=0.000), whereas pH in PMC increased (6.38±0.38, 6.40±0.62, 7.22±0.42 in layer 1, 3, 5 respectively, F=29.23, *p*=0.000). Horizontally, in all layers, pH in PMC was higher than that in PP and PMP. In deeper layers, the maps show decreasing pH with

increasing distance from the calcareous sediments. The high pH levels in calcareous sediments were derived from high inorganic carbon (10.03±2.01%), mainly $CaCO_3$.

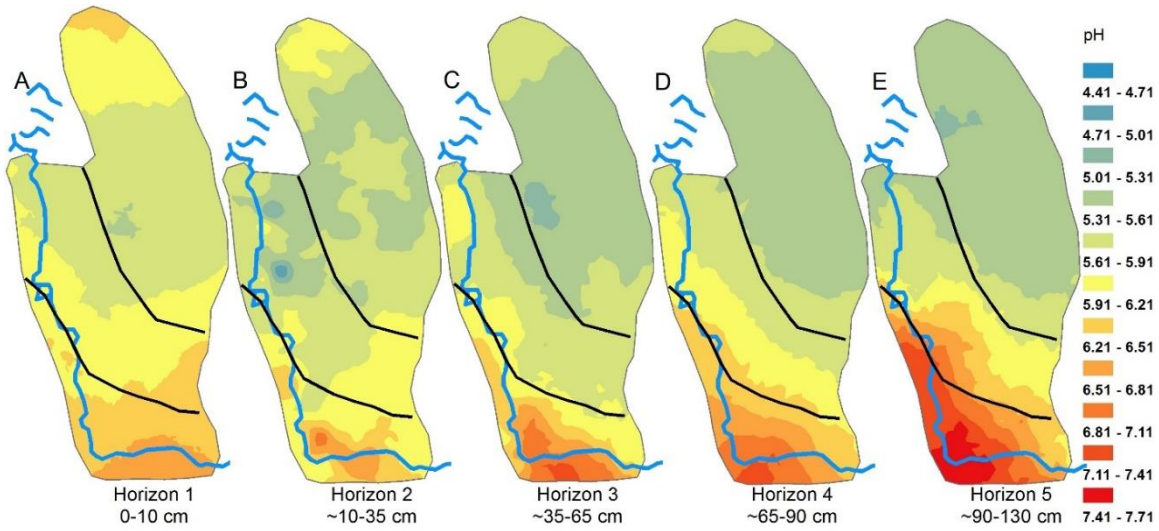

Fig. 3.5. Spatial distribution of pH in each of the five horizons; average depths of upper and lower boundaries are also shown for each horizon.

Spatial dependence (SPD) and spatial ranges were different in peat and mineral horizons. Spatial dependence was above 90% for almost all layers (Table 3.3), suggesting that the measured soil properties have high spatial dependency (>75%). SPD of θv and TN in PMP and TOC and TN in PMC in horizon 4 were smaller, suggesting modest spatial dependency (25-75%) of these soil properties in these layers and soil types. The minimum distance to obtain independent samples was 50 m in PMC, 60 m in PMP and 70 m in PP (Table 3.3).

Table 3.3. Spatial parameters of measured soil properties.

	Horizon	PMC†				PMP				PP			
		Range‡ (m)	SPD§	r^2	MD¶	Range (m)	SPD	r^2	MD	Range (m)	SPD	r^2	MD
θg	1	42.1	99.96	0.90	Gau	55.5	97.68	0.88	Gau	38.6	99.92	0.80	Gau
	2	31.5	99.95	0.79	Gau	60.4	61.56	0.84	Gau	NA	NA	NA	NA
	3	46.5	85.68	0.86	Gau	29.2	99.96	0.70	Gau	74.5	99.84	0.90	Gau
	4	NA	NA	NA	NA	32.8	50.85	0.49	Exp	67.1	99.86	0.90	Gau
	5	31.9	99.73	0.86	Gau	45.0	67.59	0.50	Gau	40.8	99.96	0.61	Gau
pH	1	25.5	85.39	0.32	Exp	NA	NA	NA	NA	43.0	99.95	0.88	Gau
	2	24.7	99.95	0.56	Gau	12.6	99.94	0.35	Gau	37.7	99.85	0.23	Sph
	3	37.5	99.77	0.79	Gau	24.6	83.78	0.46	Exp	62.1	99.98	0.90	Exp
	4	52.6	84.93	0.92	Gau	25.6	77.82	0.22	Sph	56.1	95.86	0.74	Gau
	5	99.5	78.10	0.94	Gau	12.2	97.21	0.42	Gau	59.4	68.73	0.77	Gau
TN	1	43.3	98.12	0.27	Sph	19.6	99.94	0.82	Gau	39.4	99.90	0.72	Gau
	2	24.2	99.82	0.59	Gau	13.1	98.24	0.72	Gau	33.4	98.87	0.76	Exp
	3	45.8	93.67	0.78	Gau	20.6	88.68	0.79	Exp	44.0	91.46	0.20	Sph
	4	47.1	50.07	0.45	Exp	40.0	54.07	0.60	Exp	46.3	86.33	0.77	Gau
	5	31.3	99.97	0.84	Gau	58.8	84.80	0.91	Gau	41.4	99.92	0.74	Gau
TOC	1	40.0	94.98	0.34	Sph	14.8	99.70	0.75	Gau	42.1	99.56	0.66	Gau
	2	24.5	99.92	0.59	Gau	19.2	99.71	0.83	Gau	49.7	99.88	0.88	Gau
	3	46.9	98.68	0.64	Gau	21.7	86.86	0.34	Exp	37.1	99.92	0.85	Gau
	4	45.0	50.04	0.24	Exp	25.8	88.67	0.59	Exp	11.8	99.92	0.82	Gau
	5	30.5	99.78	0.71	Gau	59.1	77.67	0.74	Gau	35.4	99.81	0.68	Gau

† PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.
‡ Range: indicates the distance of spatial dependence.
§ SPD: Spatial dependence.
¶ MD: Model used to fit semivariogram; Exp, Exponential Model; Gau, Gaussian Model, Sph, Spherical Model.

3.5.2 Ordination analysis for peat samples

Samples from all PP layers clustered together on the NMDS plots (Fig. 3.6) whereas samples from PMC and PMP were scattered along axis 1. This indicates soil properties from PP are more similar to each other compared with those from PMC and PMP. Specifically, the samples from middle peat of PMC and PMP scattered on both sides of axis 1, whereas most surface peat of PMP and most points of PP clustered on the negative side of axis 1. This indicates that surface peat of PMP and peat of PP shared similar soil properties and had low variation, whereas soil properties from middle peat of PMC and PMP showed high variability.

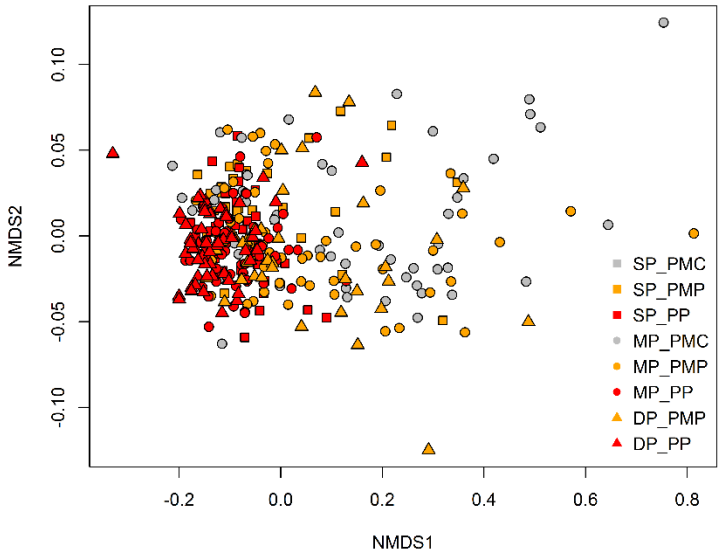

Fig. 3.6. Nonmetric multidimensional scaling (NMDS) analysis of soil properties, stress = 0.039; PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat; SP: surface peat, MP: middle peat, DP: deep peat, M: silty mineral sediment, C: calcareous sediments.

3.5.3 Pool size changes during incubation and lag phase of N dynamics in deeper peat

The basic physical and chemical properties of the eight selected soil samples in each layer of three soil types are shown in Table A.3. Soil NH_4^+ and NO_3^- concentrations measured on day 0, 7, 28 and 63 showed different trends with incubation time (Fig. 3.7). In surface peat, [NH_4^+] decreased significantly as the incubation proceeded, while [NO_3^-] increased over time (p<0.05). In middle peat, [NH_4^+] did not change until day 63 when there was a drop to about 20% of that on day 0 (p=0.000). In contrast, [NH_4^+] in deep peat increased gradually during day 0-7 (p=0.022) and day 7-28 (p=0.004), but stayed relatively stable after that. In addition, we noticed that [NO_3^-] in middle peat and deep peat started to increase after 28 days of incubation.

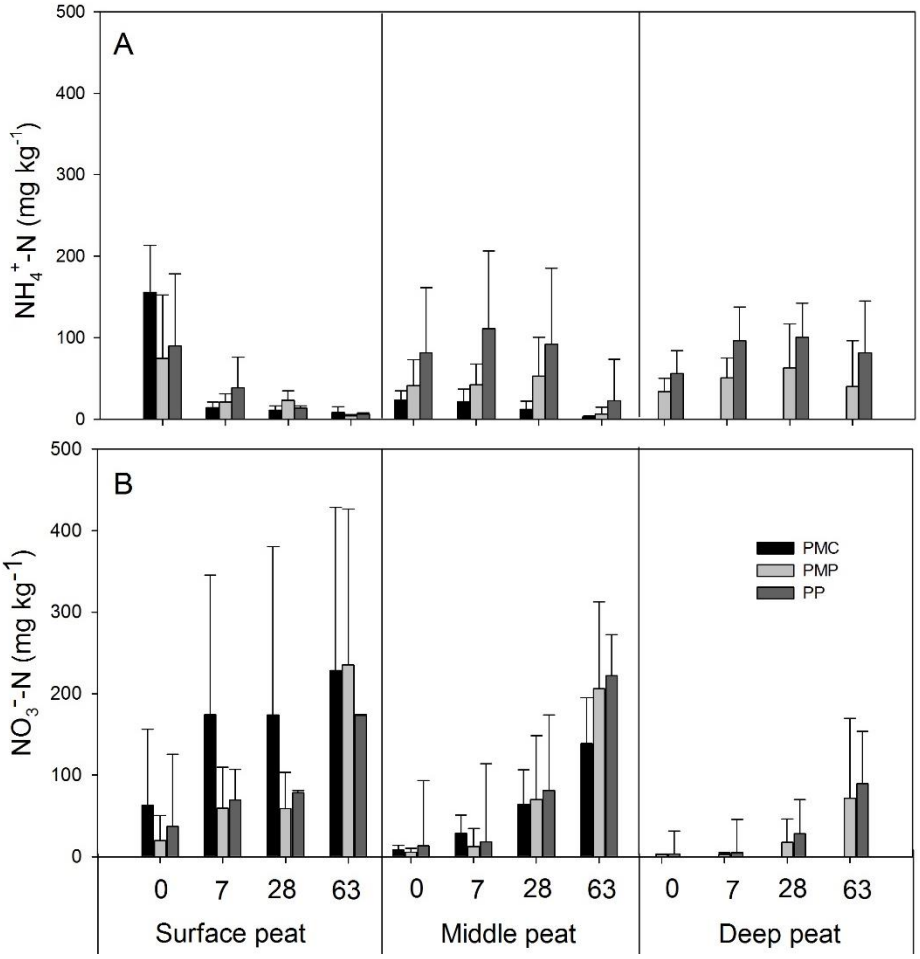

Fig. 3.7. Changes of [NH_4^+] (A) and [NO_3^-] (B) with incubation time (day 0, 7, 28 and 63) in peat samples from different depth of three soil types. PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

As subsurface peat showed a lag phase of [NH_4^+] decreasing and [NO_3^-] increasing, N cycling processes were also measured over day 0-7, day 7-28 and day 28-63 to evaluate whether there were lag phases of N cycling in subsurface peats. Nitrogen cycling processes in surface peat were the inverse of that in middle peat and deep peat under the effect of incubation time. As shown in Fig. 3.8, R_{amm} increased in surface peat from -11.70±7.34 mg kg^{-1} d^{-1} to nearly 0 mg kg^{-1} d^{-1} from day 0-7 to day 7-28 (p=0.007) and then did not change significantly thereafter. However, R_{amm} in both middle peat and deep peat went from positive to negative from day 0-7 to day 7-28 and kept decreasing to day 28-63 (p=0.000). This suggested that NH_4^+ was immobilized immediately in surface peat, but in middle peat and deep peat immobilization did not occur until after 7 days. The effect of time in different depths on R_{nit} was inverse that of R_{amm}: R_{nit} in surface

peat dropped significantly with time (p=0.003), whereas in middle peat and deep peat, R_{nit} increased with time (p=0.000). This suggested that in surface peat, the majority of NH_4^+ was nitrified to NO_3^- within 7 d; however, in middle peat and deep peat, nitrification mostly took place after 7 d of incubation. In addition, although there was no significant influence of soil types (p>0.05), a strong interaction between soil types and incubation time was found (p<0.05) except for R_{nit} in surface peat, which suggested the lag phases of N cycling were different in different soil types. For example, the lag phase of R_{amm} only achieved a significant level in PP in middle peat and deep peat, and in middle peat there was barely any lag phase of R_{nit} in PMC.

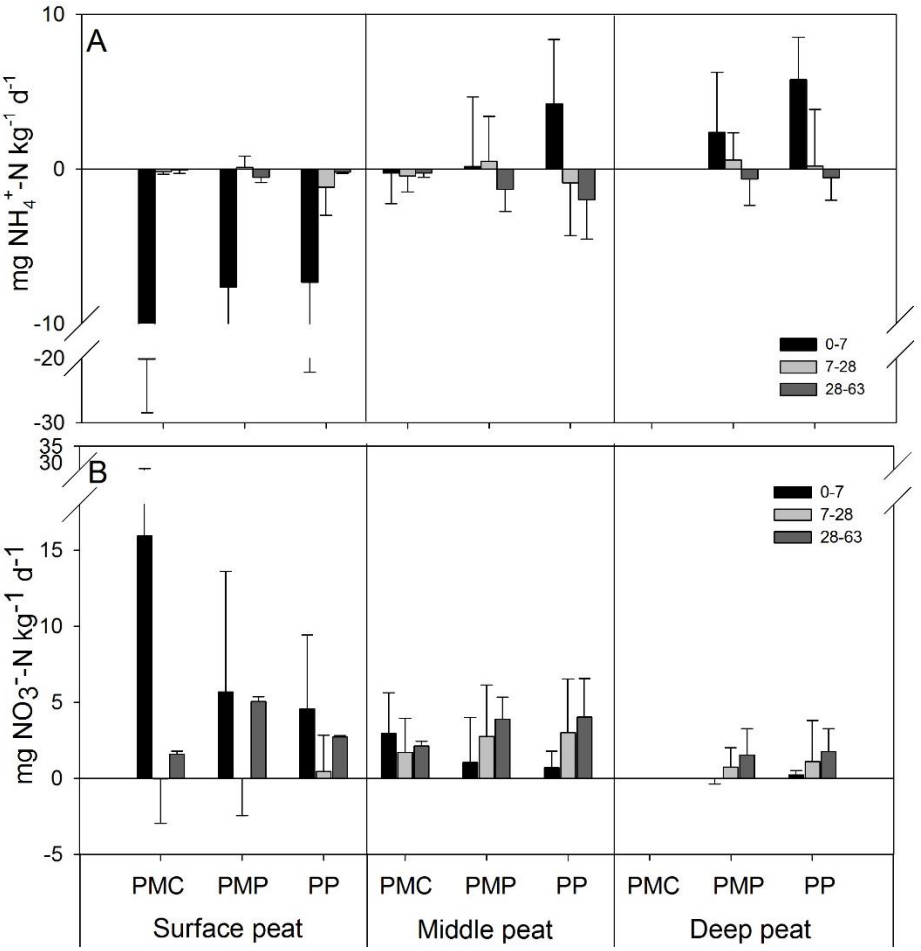

Fig. 3.8. Daily net ammonification (A) and nitrification (B) rates during day 0-7, day 7-28, and day 28-63 from surface peat, middle peat, and deep peat. PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

3.5.4 Ammonification and nitrification: net 28 d

After evaluating the effect of incubation time (i.e., to ascertain the best incubation length to avoid over- or under-estimation), N cycling processes during day 0-28 were selected to analyze effects of soil types and depth classes. Net ammonification rates (R_{amm}) were negative in surface peat and lower than that in middle peat and deep peat (p=0.000) (Fig. 3.9A). Among soil types, R_{amm} in PMC were lower than in PMP or PP (p=0.015). Net nitrification rates (R_{nit}) in deep peat during 0-28 d were about 2.2 times lower than surface peat and middle peat (p=0.000). Among soil types, R_{nit} in PMC were slightly higher than in PMP or PP (p=0.072).

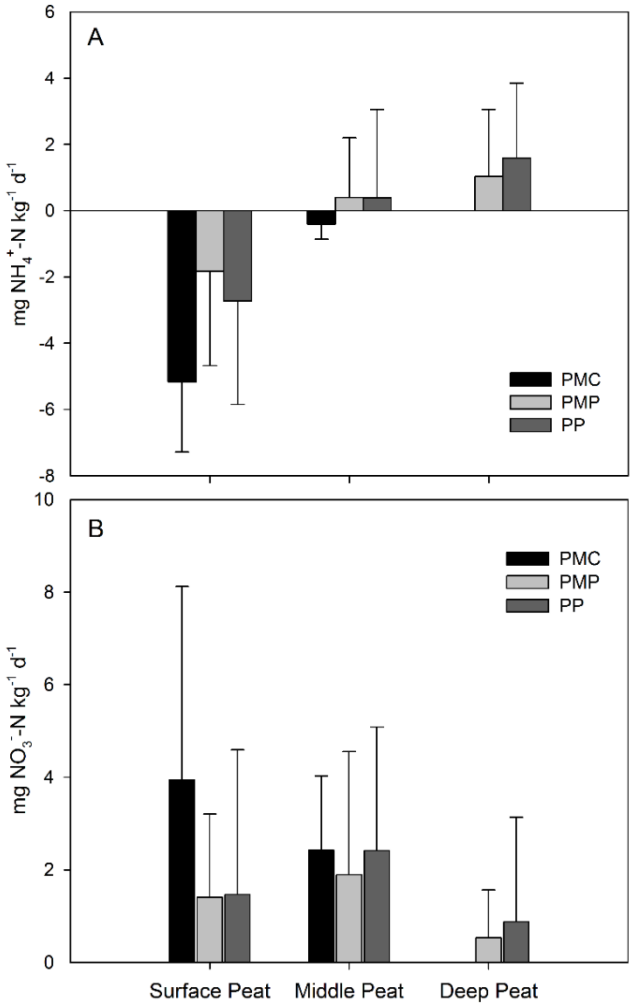

Fig. 3.9. Daily net ammonification (A) and nitrification (B) rates during 28 days from surface peat, middle peat, and deep peat. PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

To explore the potential factors affecting N cycling, the correlations between N cycling rates and soil properties were analyzed (Table 3.4). Net ammonification rates (R_{amm}) were negatively correlated with pH, initial [NH_4^+], and initial [NO_3^-] and positively correlated with C/N. In contrast, R_{nit} was positively correlated with pH, initial [NH_4^+], initial [NO_3^-] and negatively correlated with C/N. Total N, TOC and θg were not correlated with any N process.

Table 3.4. Correlations between ammonification and nitrification during day 0-28 and soil properties in peat samples.

	θg	**pH**	**TN**	**TOC**	**Initial NH_4^+**	**Initial NO_3^-**	**C/N**
R_{amm}	0.046	-0.354**	-0.031	0.163	-0.672**	-0.269**	0.238*
R_{nit}	-0.126	0.225*	0.151	-0.069	0.438**	0.390**	-0.271**

*,**, Significance at $p \leq 0.05, 0.01$, respectively.

3.6 Discussion

3.6.1 Factors regulating spatial distribution of soil properties at surface vs. at depth

Hydrology is a key factor regulating biogeochemical processes in terrestrial ecosystems, especially in wetlands (McLatchey and Reddy, 1998). Vertically, θv in layers 3 to 5 differed with vs. without mineral sediments (PMP vs. PP): θv did not increase with depth when mineral sediments were present. The mineral sediment, which has lower hydraulic conductivity (Janzen and Westbrook, 2011), slows water infiltration; therefore, water accumulates on top of mineral sediments and flows horizontally towards the creek rather than vertically to the underlying peat. Thus, as expected, the presence of mineral sediments influenced θv spatial distribution in adjacent deeper layers but not in upper layers, which will be discussed later.

The types of mineral sediments regulated pH, which regulated biogeochemical processes via controls on microbial activity. Approximately 80% of the surface horizons in this basin were characterized by pH levels in the range associated with poor fen to moderately rich fens (Sjörs and Gunnarsson, 2002). In fens, pH typically decreases with depth (Vitt et al., 1995) because dissolved organic acids formed from more decomposed upper layers flush down to deeper locations in the soil profile by seasonal fluctuations in water table. This is consistent with PP profiles, which were located further from calcareous sediments. Also, in both PMC and PMP, pH in the middle peat was typically lower than in surface peat. However, below that, pH increased dramatically in PMC and PMP with depth due to the proximity to calcareous sediments. Because

of high $CaCO_3$ content, calcareous sediments had higher pH (7.39±0.11) compared to silty mineral (5.96±0.33) or peat (5.81±0.44) (p=0.000). Calcareous sediments are usually found in rich fens (Chadde et al., 1998) owing to upward flow of mineralized groundwater into the peat profile (Glaser et al., 1996).

Water content and pH are parameters known to affect decomposition rates (Blodau, 2002), which in turn affect TOC and TN. Total OC and TN shared similar patterns, as most N was stored in the organic form. The increase of TOC with depth in PP is consistent with other studies in poor fen and *Sphagnum* bog peatlands (Belyea and Malmer, 2004; Cocozza et al., 2003). Middle peat and deep peat in PP are originally from *Sphagnum* moss which is a strong proton donor, and therefore a strong acidifier (Aerts et al., 1999). In peat profiles lacking mineral horizons, these compounds would leach downward, accumulate in deeper peat layers, and slow down decomposition (Vitt, 2006). However, our results show TOC decreases with depth in PMC and PMP. In PMC, this is because the middle peat origin in PMC is sedge which is more readily decomposed than *Sphagnum* mosses, as discussed. In addition, carbonates in PMC neutralize organic acids and raise the pH of peatland to near neutral, which favors microbial decomposition of SOM thus lowers the TOC content. Previous research also found that richer fens showed higher microbial activity and lower peat accumulation (Moore and Basiliko, 2006). In PMP, deep peat had lower TOC than PP because of different decomposition rates and microbial activity controlled by the stratified mineral horizon. It is possible that decomposition rates and microbial activity are higher in deep peat in PMP with lower θv, as drier conditions encourage decomposition. Overall, mineral sediments mainly influenced TOC and TN spatial distribution in deep layers via influencing θv, leaching controlled by groundwater movement and pH.

Stratified mineral horizon was not the only influence on soil properties in this peatland. Also contributing to high spatial variation of measured soil properties in the upper soil layer was the presence of Bateman Creek. Water content and TOC were lower in locations near the creek where the elevation is lower (Fig. 3.2 and 3.3). Others have shown that the presence of stream channels in peatlands lowers the water table in riparian zones (Patterson and Cooper, 2007). Under a lowered water table, surface peat becomes unsaturated and the oxygen availability increases. This results in enhanced peat decomposition (Chimner and Cooper, 2003). Unlike in lower layers (Fig. 3.5C-E) where pH consistently decreased from southwest to northeast, the lowest pH level in upper layers (Fig. 3.5A&3.5B) appeared in the middle part of the peatland.

The higher pH of southwest resulted from the carbonates in soil profile, as pH increased with depth. However, the higher pH (pH>6.0) in the northeast and southeast of peatland decreased with depth. It is possible that pH in the northeast was affected by the lateral flow of East Inlet, which not only flushes away organic acids but also introduces groundwater with higher pH (pH=8.06) from surrounding mountains into the peatland; the higher pH in the southeast corner might have been due to the beaver pond in the southeast corner, which collects lateral inflows from the hillslopes and precipitation.

3.6.2 Effects of stratified mineral horizon and depth classes on N cycling

N cycling was indirectly regulated by mineral sediments via pH. The pH level in peat samples ranged from 5.41 to 6.27, which is within a favorable range for microbes (Sjörs and Gunnarsson, 2002). In addition, pH was positively correlated with nitrification and negatively correlated with ammonification. Previous studies have declared that high acidity may inhibit nitrification when pH is lower than 5.5, because ammonia oxidation and nitrite oxidation release H^+ (Aciego Pietri and Brookes, 2008; Bridgham et al., 1998). Therefore higher pH promotes nitrification, which results in lower [NH_4^+]. In contrast, low pH has been found to increase [NH_4^+], because less [NH_4^+] will be nitrified, and thus result in [NH_4^+] accumulation and higher net ammonification (Aciego Pietri and Brookes, 2008). In our 28-d incubation, higher pH deriving from the underlying calcareous sediments in PMC promoted R_{nit} but lowered R_{amm}. In PMP and PP, where pH was mostly lower than 6, especially for deeper *Sphagnum* moss peat, acid-tolerant bacteria may be involved in nitrification (De Boer and Kowalchuk, 2001). Moreover, mineral sediments might also influence N cycling in other ways. For example, the mineral sediments in PMC and PMP block water infiltration and provide more Fe^{3+} and Mn^{4+} which could serve as electron acceptors and help convert NH_4^+ to NO_2^- and then reduced to N_2 via anammox-coupled nitrification-denitrification (Clément et al., 2005; Pavlekovic et al., 2009). More research should be done to study the possibility of Fe and other elements interacting with N cycling with presence of mineral sediments.

Depth influences N cycling via decreasing O_2 availability, microbial activity and changing substrates along a soil profile (Preston et al., 2012). Therefore, N cycling rates are expected to decrease with depth (Iversen et al., 2011; Persson and Wirén, 1995). In this research, during day 0-28 incubation, ammonification rates in surface peat were negative. This is due to NH_4^+

consumption by nitrification and/or immobilization, which are promoted by high microbial biomass and activity at surface (Preston et al., 2012). In middle peat and deep peat, less inorganic N was immobilized or nitrified because of less available O_2 and lower microbial biomass and activity in deeper layers. In addition, with different origins of peat, C/N ratios were different at depth. Ammonification is often negatively related to C/N ratio (Bayley et al., 2005), but the C/N ratios at this site showed positive correlations with net ammonification rates, as seen by Updegraff et al. (1995). One possibility for this positive relationship is that *Sphagnum* peat can be divided into a labile N pool and a recalcitrant N pool, where the former has higher N mineralization rates and the latter has lower N mineralization rates (Limpens et al., 2006). In our studies, the C/N ratio was higher in deep peat of PP and PMP (PP especially); deep peat originates from *Sphagnum* moss and is more fibric compared with surface peat. Therefore, deep peat might have more labile N and when incubated at surface-like conditions, showed a higher N mineralization rate.

Interestingly, a lag phase of N cycling was found in deeper layers due to effects of depth: i.e., lower O_2 availability, microbial biomass and activity and pH. In surface peat, NH_4^+ was largely converted to NO_3^- during day 0-7. Also, as in middle peat and deep peat, NH_4^+ accumulated at the beginning of incubation, and then started to decrease or stay the same when nitrification or immobilization increased. One possible reason is that microbial biomass in deeper peat can be significantly lower than that in upper peat, and at greater depths the microbial activity has been found to be very low (Preston et al., 2012). Also, although incubated in surface condition, microbes from deeper peat might need time to adapt to surface conditions, i.e., higher temperature and more aerobic condition (Brune et al., 2000; Schmidt et al., 2002). In addition, upper peat layers and lower peat layers might be dominated by different nitrifiers: acid-sensitive nitrifiers are relatively important in upper organic layers, whereas acid-tolerant nitrifiers are important in lower organic layers. Previous work has found that the production of NO_3^- by acid-tolerant bacteria increased faster after 2 weeks of incubation than during earlier incubation stage, which is consistent with our finding, i.e., the lag phase of nitrification in deep peat of PP and PMP, which originated from *Sphagnum* moss and had lower pH (De Boer et al., 1989).

For the interaction of lag phase and soil types, one possibility was that the higher pH derived from calcareous sediments mitigated the lag phase. In middle peat, R_{nit} in PMC did not increase with incubation time, R_{nit} in PMP showed increasing pattern (but not significantly),

while R_{nit} in PP significantly increased (Fig. 3.8B). As described above, nitrification may have been promoted by higher pH in PMC; this may have reduced the time that microbes needed to adapt to surface conditions. Alternatively, the higher pH may have benefited the growth and activity of acid-sensitive nitrifiers (Persson and Wirén, 1995), which might be dominant in middle peat of PMC. Similarly, R_{amm} in middle peat for PMC was negative at the beginning, as higher pH promotes nitrification, which results in lower [NH_4^+] (Aciego Pietri and Brookes, 2008).

3.7 Conclusion

Spatial distributions of key soil properties (TOC, TN, pH and θv) in this mountain peatland showed remarkable variability with soil types affected by mineral sediments. Stratified mineral horizon regulated spatial distribution of pH by providing carbonates to some layers, and influenced θv via slowing water infiltration into deeper layers. These affected organic matter decomposition and thus TOC and TN distribution. However, the influence of mineral material did not extend to the two upper layers. In addition, stratified mineral horizon also affected N cycling processes in this peatland by affecting the studied soil properties and organic matter decomposition. In particular, higher pH where calcareous sediment was present highly promoted nitrification. Depth also affected N cycling processes with the changes of peat origins, O_2 availability, and listed soil properties. We not only found the differences in N cycling processes rate changes with depth, but also a lag phase for N cycling in deeper layers. Moreover, the lag phase was also affected by mineral sediments. Based on the above, it appears that the presence of mineral sediments in mountain peatlands can influence the spatial distribution of soil properties and further affect biogeochemical processes. More study is needed on biogeochemical processes in complex peatland soil profiles. In particular, it would likely be fruitful to explore distributions of microbiological composition to better understand what driving change in biogeochemical processes is in these complex peat-mineral systems.

4. Assessing Pedogenic Controls on Carbon Mineralization, Organic Matter Composition and Microbial Community Dynamics in A Mountain Peatland[1]

4.1 Preface

Chapter 3 showed that the presence and types of stratified mineral horizons regulate movement of groundwater and diffusion of major and trace nutrient elements with groundwater, which affect spatial distribution of soil properties and N cycling rates. As carbon cycling and microbial community structures are correlated with these soil properties, it is highly likely that stratified mineral horizon may influence C cycling and microbial communities. This chapter examines whether mineral horizon influence C cycling, associated peat chemistry, and microbial community structures.

[1] Wang, X., Helgason, B., Westbrook, C., & Bedard-Haughn, A. (2016). Effect of mineral sediments on carbon mineralization, organic matter composition and microbial community dynamics in a mountain peatland. Soil Biology and Biochemistry, 103, 16-27. Xiaoyue Wang is the major contributor and author of the manuscript. Bobbi Helgason is the committee member and helped with microbial data analyses and writing. Cherie Westbrook is the committee member and helped with experimental design, sampling and writing. Angela Bedard-Haughn is the supervisor and helped throughout this study.

4.2 Abstract

Carbon (C) dynamics in northern peatlands are an important factor in the global C balance under climate change scenarios. The C dynamics are microbially driven and influenced by the chemical composition of organic matter. Peatlands in the Rocky Mountains are usually formed on mineral sediments or developed with interbedded mineral lenses, which have been found to affect soil properties such as volumetric water content, pH, TOC and TN. Our objective was to investigate whether the presence and relative depth of mineral horizons (i.e., stratified mineral horizons) affect microbial community structure and C composition, and in turn influence C mineralization. Three organic soil profile types were selected in the Sibbald research wetland of southwestern Alberta: peat over silty mineral over calcareous sediment (PMC), peat over silty mineral over peat (PMP), and sedge peat over moss peat profiles (PP). Peat samples were subjected to C composition and microbial community abundance and structure measurement and then incubated to test potential C mineralization. The main differences were detected in subsurface peat. In subsurface peat above mineral sediments (PMC, PMP) versus at equivalent depth in PP, the presence of a mineral horizon caused different C mineralization (mg C-CO_2 kg^{-1} soil) among soil types (PP > PMC and PMP). In addition, specific C mineralization (mg C-CO_2 kg^{-1} SOC) decreased with depth in subsurface peat in PP, but not in PMP, as greater volumetric water content (θv) above the mineral horizon created anaerobic conditions in PMP. Microbial community structures also differed between PMP and PP due to different θv in peat below mineral sediments. Recalcitrant C: labile C, bacteria: fungi, and microbial physiological stress were greatest in the subsurface peat above mineral sediments. Depth had an even greater effect: both C mineralization and microbial abundance decreased significantly with depth. Moreover, microbial community structure mainly grouped according to relative depth. Overall, our findings indicated that stratified mineral horizons affected C mineralization, microbial community structure, and peat chemistry in subsurface peat.

4.3 Introduction

Northern peatlands play an important role in global C cycling, as they constitute approximately 30% of global soil carbon (C) and have the greatest organic carbon (SOC) density (1140-1430 Mg C ha^{-1}) with only 3% coverage of the land area (Eglin et al., 2010; Gorham, 1991). High C density and C accumulation in these cold, waterlogged peatlands indicate the

potential for substantial CO_2 and CH_4 emission (Frolking et al., 2011). Northern peatlands have been acting as a C sink, but the response of the global net C balance to a changing climate remains uncertain (Wu and Roulet, 2014). Moreover, peatland stratigraphy can be complex with great variety in peat thickness, origin, and hydrochemical properties (Charman et al., 1995). In the Rocky Mountains, for example, peatland stratigraphy can be considerably more complex owing to regional geomorphic instability. Peat profiles in mountain environments can include frequent interruptions by mineral or ash lenses (Kubiw et al., 1989; Sewall et al., 2015) or contain underlying mineral sediments (Chadde et al., 1998; Kubiw et al., 1989; Morrison et al., 2015) originating from upslope slides. Little is known about whether and how mineral sediments affect C cycling in peatlands.

Climate factors such as temperature and water content are the dominant controls of C mineralization (Preston and Basiliko, 2016; Sierra et al., 2015). Carbon dynamics are further influenced by organic matter (OM) stability (Davidson and Janssens, 2006; Six et al., 2002). The OM stability depends on whether OM is protected from microbial decomposition by physical (aggregates), chemical (organo-mineral complexes and/or adsorbed by clay or silt) and/or biochemical (OC composition) mechanisms (Han et al., 2016). In peatlands, biochemical protection is key because bulk peat is usually weakly decomposed and unprotected by mineral particles. Biochemical stability is reflected in the relative abundance of labile versus recalcitrant C functional groups (Beer et al., 2008). As peat decomposes, OM composition changes, with increasing recalcitrant C and decreasing labile C (Tfaily et al., 2014). Different residues have different OM compositions: Mosses (such as *Sphagnum* spp.) are more likely to release phenolic compounds whereas vascular plants are more likely to release lignin (Verhoeven and Toth, 1995; Williams et al., 1998). Fourier Transform Infrared (FTIR) spectroscopy has been widely used in peatlands (Artz et al., 2006; Broder et al., 2012; Tfaily et al., 2014) as it provides absorption bands characterizing the relative abundance of C compounds (i.e. polysaccharides, phenolic and aliphatic, aromatic groups, and lignins) via ratios of recalcitrant fractions and labile fractions (Beer et al., 2008). However, it remains unclear how these C fractions affect C mineralization in peatlands, particularly in the presence of complex stratigraphy.

Microbes are important mediators of C mineralization. Microbial community dynamics change with nutrient levels, pH, and especially O_2 availability, which is often associated with increasing depth and/or water content related to hydrologic conductivity (Artz et al., 2006; Fierer

et al., 2003; Lin et al., 2014). Microbial community composition and peat chemistry are highly correlated with each other. The chemical composition of organic matter is affected by microbial decomposition (Šnajdr et al., 2011); however, different C fractions also support different microbial communities (Preston et al., 2012). For example, microorganisms can be divided ecologically into copiotrophs and oligotrophs (Fierer et al., 2007). Copiotrophs prefer soils with more labile C, whereas oligotrophs are usually more abundant in soils with higher content of recalcitrant C (Bastian et al., 2009). Phospholipid fatty acid (PLFA) analysis is widely used to monitor broad changes of the viable microbial community structures with C decomposition and sensitivity of the microbial community structure to substrate quality and other abiotic factors (Peltoniemi et al., 2015; Preston et al., 2012). It has been found that the shift of microbial community structures from copiotrophs to oligotrophs as a result of different C composition can be reflected by increasing gram positive bacteria to gram negative bacteria ratio (GP:GN) in PLFA measurement (Yao et al., 2000). In addition, some GN bacteria with cyclopropyl fatty acids in their membrane respond to stress associated with nutrient depletion, O_2 status, acidic pH, and osmotic stress (Grogan and Cronan, 1997). Therefore, the ratio of cyclopropyl fatty acids to monounsaturated fatty acids is used as an indicator of physiological stress. Moreover, bacteria to fungi ratios (B:F) reflect soil function and vary with environmental conditions such as temperature, O_2, pH, nutrients, and available C (Prosser et al., 2007).

In peatlands with complex stratigraphy, mineral sediments can provide nutrients and electron acceptors, which can increase decomposition of nearby peat (Broder et al., 2012). Our previous work showed that the presence and types of stratified mineral horizons regulated movement of groundwater and diffusion of major and trace nutrient elements with groundwater, which affected spatial distribution of soil properties such as volumetric water content (θv), pH, total organic C (TOC) and total nitrogen (TN) (Wang et al., 2016b). Changes in these soil properties should impact peat profile development, microbial communities and biogeochemical processes (Lehmann and Kleber, 2015), but this requires investigation. We conducted this study to: 1) examine whether the stratified mineral horizons affect C mineralization by influencing microbial community structure and C composition; and 2) determine whether the influence of stratified mineral horizons varies by depth.

4.4 Material and Methods

4.4.1 Sites description

Sibbald Research Wetland in the Kananaskis region of southern Alberta, Canada was selected as the research site (Latitude: 51.06N, Longitude: 114.87W) (Fig. 4.1A). This hummocky peatland is located within a relatively level valley at 1480 m a.s.l. The peat origins in this peatland are sedges (*Carex aquatilis*; 40-50% fiber content) for upper peat (~0-50 cm). Deeper peat (~50-130 cm) was dominated by less decomposed mosses (60% fiber content), mostly *Sphagnum* spp. mosses and a few brown mosses (*Drepanocladus sp. and Scorpidium sp*). The site has been described by Janzen and Westbrook (2011) and Westbrook and Bedard-Haughn (2016). We classified the research area into three main soil types according to the presence and type of stratified mineral horizons (Wang et al., 2016b). Briefly, in the southwest zone of the basin, with the presence of silty mineral and calcareous sediments at the base, the soil profile is sedge peat/silty mineral sediment/calcareous sediment (PMC). In the central zone, a silty mineral horizon is interbedded in peat, i.e. sedge peat/silty mineral sediment/moss peat (PMP). In the northeast zone, no mineral horizons are present within 2 m, so it is classed as sedge peat/moss peat (PP) (Fig. 4.1B). Sedges are the most common vegetation in PMC and along the creek in PMP. In PP and the remainder of PMP, both sedges and willows (*Salix spp.*) are dominant. For PMC and PMP profiles, each can be further divided into two sub-types according to depth of mineral lens (Fig. 4.1C). PMC1 and PMP1 represent profiles where mineral horizons or mineral lenses are present at shallower depth (30-40 cm), whereas PMC2 and PMP2 represent the profiles where mineral horizons or mineral lenses are present at greater depth (>50 cm). In total there are five sub-types: PMC1, PMC2, PMP1, PMP2 and PP.

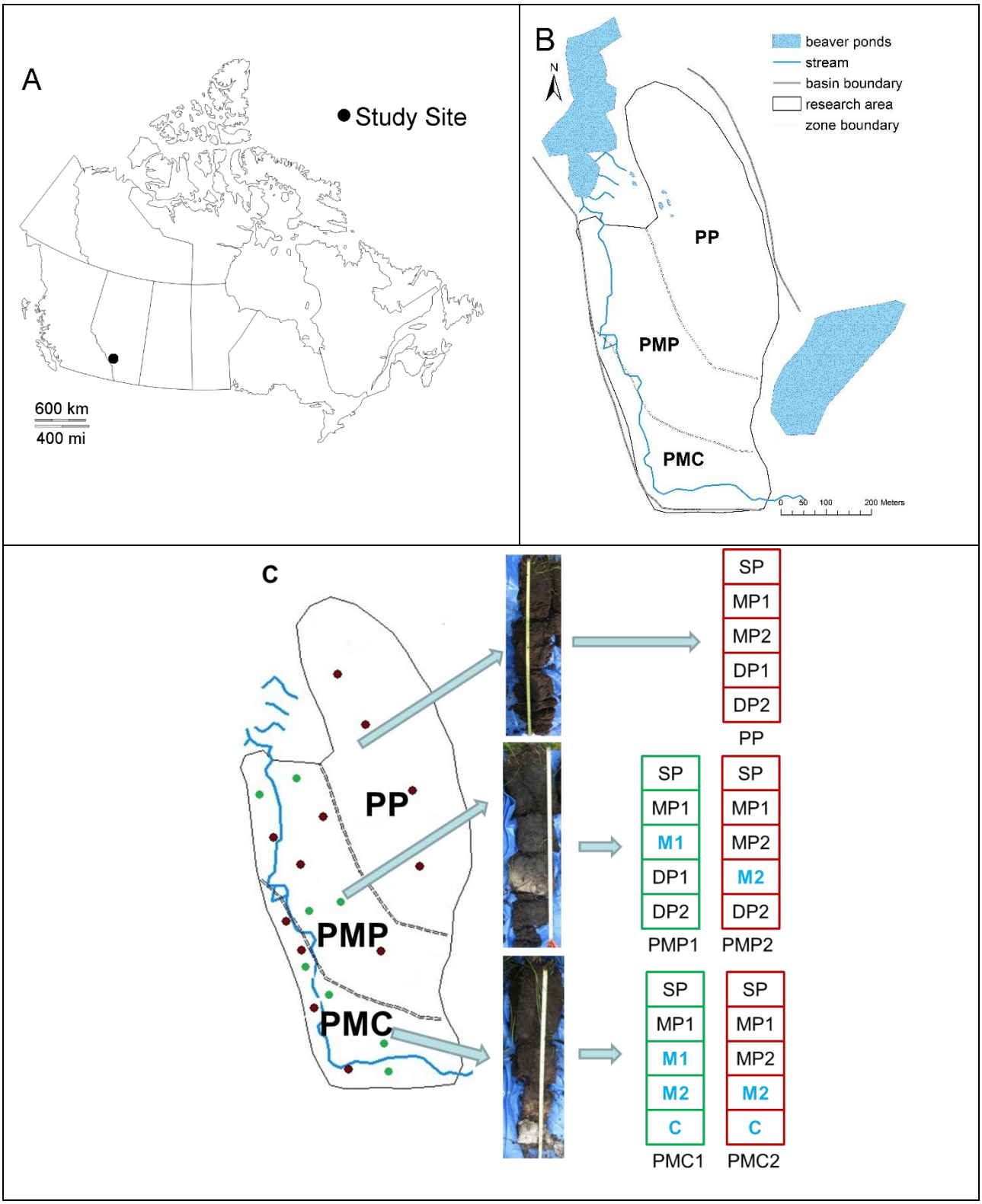

Fig. 4.1. A) Location of the field site. B) Research area and soil types according to soil profile stratified mineral horizon. C) Sampling points, soil types and sub- types from each zone, the colors of the sampling points associated with colors of the column. SP: surface peat; MP: middle peat; DP: deep peat; M: silty mineral sediments; C: calcareous sediments.

Eight sampling points were selected from each soil type (including four from each of the PMC and PMP sub-divisions). The distances between any two given sampling points were greater than the minimum distance to obtain independent samples (50 m in PMC, 60 m in PMP and 70 m in PP; Wang et al., 2016). At each sampling point, samples were collected by auger to approximately 1.3 m depth, including equal thicknesses of peat above and below the silty mineral sediments in PMP and including the carbonate sediments at the base of PMC. At each sampling point, the peat profile was divided into five layers according to depth and stratified mineral horizons. The first layer was the top 10 cm; below that, layers were divided according to transition of mineral sediment and peat, changes of fiber content according to von Post test, and thickness (Wang et al., 2016b). To simplify the depth of peat relative to mineral sediments, peat materials were grouped into three depth classes: *Surface peat* referred to the top 10 cm, peat layer(s) above mineral sediments was *middle peat* (~10-65 cm), and peat layers below mineral were *deep peat* (~65-130 cm). Deep peat was only measured in PMP and PP; there was no deep peat in PMC. These five layers were named according to depth classes and relative depth; for example, if layer 2 and layer 3 are both middle peat, then layer 2 was *middle peat 1 (MP1)* and layer 3 was *middle peat 2 (MP2)*. The five layers in different sub-types are named differently as shown in Fig. 4.1C; for example, in PP, the five layers are surface peat/middle peat1/middle peat2/deep peat1/deep peat 2 (SP/MP1/MP2/DP1/DP2); in PMP1, the five layers are surface peat/middle peat1/silty mineral sediments1/deep peat1/deep peat 2 (SP/MP1/M1/DP1/DP2). Ethanol was used to sterilize the auger between samples. Samples were stored frozen (-80°C) until analysis. The basic soil properties were measured or described (Wang et al., 2016b) and are summarized in Table 4.1. Each sample was divided into three sub-samples for C mineralization, C composition and microbial community abundance and structure measurements.

Table 4.1. Basic soil properties in five soil sub-types.

Sub-types†	Layers‡	θv (cm^3 cm^{-3})§	pH	TOC (%)	TN (%)	C:N
PMC1	SP	0.75 ± 0.24	6.31 ± 0.49	43.59 ± 4.18	2.85 ± 0.33	15.41 ± 2.25
	MP1	1.01 ± 0.37	6.16 ± 0.57	37.40 ± 7.90	2.89 ± 0.50	12.87 ± 0.87
	M1	0.61 ± 0.14	6.40 ± 0.60	8.80 ± 7.66	0.66 ± 0.61	13.57 ± 1.36
	M2	0.64 ± 0.28	6.84 ± 0.46	3.23 ± 1.57	0.27 ± 0.16	16.84 ± 5.21
	C	0.77 ± 0.13	7.38 ± 0.02	2.18 ± 0.89	0.15 ± 0.08	135.17 ± 122.2
PMC2	SP	0.52 ± 0.07	6.24 ± 0.32	36.45 ± 4.87	2.62 ± 0.36	13.98 ± 1.27
	MP1	0.75 ± 0.05	5.78 ± 0.19	32.73 ± 8.33	2.52 ± 0.56	12.93 ± 0.64
	MP2	0.80 ± 0.08	6.05 ± 0.16	22.51 ± 7.80	1.71 ± 0.54	12.75 ± 1.07
	M2	0.82 ± 0.35	7.08 ± 0.48	4.59 ± 3.01	0.29 ± 0.22	57.29 ± 50.28
	C	0.77 ± 0.14	7.42 ± 0.11	2.89 ± 0.57	0.15 ± 0.09	365.92 ± 583.53
PMP1	SP	0.86 ± 0.18	5.40 ± 0.42	38.87 ± 7.26	2.91 ± 0.34	13.38 ± 2.06
	MP1	0.72 ± 0.19	5.62 ± 0.30	35.36 ± 7.69	2.45 ± 0.44	14.35 ± 1.20
	M	0.74 ± 0.14	6.09 ± 0.33	1.51 ± 0.82	0.07 ± 0.07	12.21 ± 15.31
	DP1	0.58 ± 0.12	5.84 ± 0.27	38.67 ± 4.66	2.43 ± 0.50	16.21 ± 1.80
	DP2	0.51 ± 0.09	6.08 ± 0.77	35.61 ± 7.59	2.16 ± 0.42	16.53 ± 2.01
PMP2	SP	0.83 ± 0.19	5.42 ± 0.28	42.93 ± 5.74	2.95 ± 0.28	14.52 ± 0.96
	MP1	1.15 ± 0.18	5.26 ± 0.60	37.97 ± 5.50	2.63 ± 0.41	14.55 ± 1.98
	MP2	1.57 ± 0.43	5.68 ± 0.26	43.15 ± 2.70	2.86 ± 0.12	15.10 ± 0.71
	M1	1.26 ± 1.12	5.92 ± 0.24	4.33 ± 5.73	0.27 ± 0.43	32.66 ± 14.82
	DP2	0.64 ± 0.14	5.85 ± 0.73	37.71 ± 4.49	2.26 ± 0.28	16.78 ± 1.69
PP	SP	0.55 ± 0.07	6.04 ± 0.41	43.10 ± 0.99	2.66 ± 0.27	16.34 ± 1.63
	MP1	0.72 ± 0.13	5.68 ± 0.27	44.55 ± 2.30	3.14 ± 0.18	14.20 ± 0.94
	MP2	0.64 ± 0.11	5.62 ± 0.17	42.74 ± 2.04	2.90 ± 0.31	14.83 ± 1.25
	DP1	0.67 ± 0.16	5.48 ± 0.08	44.13 ± 0.93	2.90 ± 0.15	15.22 ± 0.53
	DP2	0.71 ± 0.15	5.38 ± 0.09	47.36 ± 2.11	2.80 ± 0.52	17.44 ± 3.91

† PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.
‡ SP: surface peat, MP: middle peat, DP: deep peat, M: silty mineral sediments, C: calcareous sediments.
§ θv was calculated from θg and bulk density, bulk density was measured from one sample in each layer of each soil type.

4.4.2 Potential C mineralization

To maintain aerobic conditions, the mass of peat (6–60 g) used for incubation was adjusted according to TOC content; less was used for samples with higher TOC (Table 4.2). Samples were incubated in 1L mason jars under surface-like condition (22°C at field moisture; Table 4.1). Gravimetric water content was monitored every other day and distilled water was added if needed to maintain field-moist conditions. After incubating for 1, 3, 5, 7, 14, 21, 28, 35, 49 and 63 d, 20 mL headspace gas samples were extracted with a syringe. Between sampling times, each jar was covered but not sealed, leaving a small opening to allow gas exchange. This opening was sealed 24 h before each sampling time (Chow et al., 2006). Before sealing, ambient air was used to flush the jars. Immediately after the lid was sealed, gas samples from 10 random jars were taken and used as a base reading. All samples were analyzed for CO_2 using gas chromatography (Bruker 450 GC, Bruker Biosciences Corporation, USA) with a thermal conductivity detector (TCD). Carbon dioxide standards of 455, 1018, 2020, 5000 and 25100 ppm were used for calibration. Data was processed via Varian MS Workstation (version 6.9.3, Mississauga, ON). Total C mineralization was calculated from the amount of C produced per unit of soil (mg CO_2-C per kg soil). Specific C mineralization was calculated from the amount of C produced per unit of total organic C (mg CO_2-C per kg SOC).

Table 4.2. Sample types and equivalent dry weight used in incubation.

Sample types	TOC (%)	Dry weight (g)
Surface Peat	40%	6
Middle Peat	30-40%	10
Deep Peat	30-40%	10
High C Silty mineral	10%	40
Low C Silty mineral	1-3%	60
Calcareous sediment	1-3%	60

4.4.3 Phospholipid fatty acid (PLFA)

Phospholipid fatty acids were determined following the method described by Helgason et al. (2010). Briefly, soil samples were freeze-dried and ground with mortar and pestle to pass through a 2 mm sieve. Approximately 5.0 g of mineral soil or 1.0 g of peat samples were weighed into 50 mL sterilized tubes and exact weights were recorded. Bligh-Dyer solutions

(MeOH: $CHCl_3$: citrate buffer=2:1:8) were used to extract fatty acids from soil samples. After extracting the supernatant, citrate buffer and $CHCl_3$ were added in a wash step. Then non-polar phases were transferred and evaporated by N_2, leaving behind only fatty acid. Then PLFAs were separated from neutral lipids and glycolipids with a silicic acid bonded solid-phase-extraction column (Varian Inc., Mississauga, ON) and samples were dried with N_2. Dried lipids were saponified and methylated to fatty-acid methyl esters (FAME). Fatty acid methyl esters were re-suspended in hexane and 10μL nondecanoic acid methyl ester (0.1 μg μL^{-1}) was added as an internal standard, then dried with N_2. Individual FAMEs were identified using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE, USA). Individual biomarkers were assessed according to Helgason et al. (2014). Specifically, bacteria biomarkers are i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0, cy19:0, while fungi biomarker is only 18:2ω6,9. Biomarkers represent gram positive bacteria were i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and those represent gram negative bacteria were 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0. Physiological stress biomarker was termed Stress, and represents the ratios of cy17:0 to 16:1 ω7c. In this study we did not find cy 19:0 fatty acid.

4.4.4 Carbon composition

Fourier transform infrared (FTIR) spectroscopy was used to determine C fractions in peat samples. Sub-samples of peat were dried, ground using a ball mill, and then placed on a disc with a diamond-coated ZnSe crystal. The spectra data were collected by a FTIR spectrometer (Bruker Optics Equinox 55, Ettlingen, Germany) connected to a $N_{2(l)}$-cooled MCT detector. A total of 256 scans were collected at 4 cm^{-1} resolution. Ambient air was used as background for all samples. Spectra data were measured over 4000 to 400 cm^{-1}; however, the range of 1800 to 400 cm^{-1} was used for data analysis, as this range shows the majority of variations and covers key C fractions including biochemical labile C and biochemical recalcitrant C. Specifically, polysaccharides were represented by absorption band at 950-1170 cm^{-1} (1033 cm^{-1}). Biochemical recalcitrant C fractions are phenolic and aliphatic structures (~1420 cm^{-1}), aromatic C=C or CO of amide groups (~1510 cm^{-1}), and lignin and other aromatics and aromatic or aliphatic carboxylates (~1630 cm^{-1}) (Broder et al., 2012). The relative peak ratios of 1630/1034, 1516/1034 and 1414/1034 were used as ratios of recalcitrant C to labile C (RC:LC).

Water-extractable organic carbon (WEOC) was determined following the method described by Chantigny et al. (2008). Fresh soil (10±0.04 g) was gently mixed with 100 mL of deionized water and incubated at 4°C for 24 h, then filtered through 0.45 µm polycarbonate membrane filter (Whatman Inc., Piscataway, NJ). The WEOC was measured using a TOC-VCPN analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Potassium hydrogen phthalate (0-200 mg L^{-1}) was used as the standard.

4.4.5 Statistical Analysis

The FTIR spectra were first subjected to baseline correction and spectra normalization and then averaged (n=4) for each layer in each sub-type. Homogeneity of variances was tested by the Bartlett test. The Shapiro-Wilk test and histograms were used to evaluate data normality. Total PLFA concentration and C mineralization rates were transformed by log10 to normalize them; other variables were already normally distributed. Analysis of variance (ANOVA) was used to determine differences in C mineralization rates, RC:LC ratios, and important ratios of microbial functional groups among sub-types and in different depth classes through fitting general linear model using the glm function in R (R 3.1.2, R Development Core Team, 2014).

Nonmetric multidimensional scaling (NMDS) was used to explore microbial community structure across the three soil types and three depth classes. Similarity analysis (ANOSIM) was used to determine the differences in microbial community structures among soil types and depth classes. The FTIR absorption band group intensities were analyzed by Permutational Multivariate Analysis (PERMANOVA) to test differences among soil types and depth classes. Similarity percentage (SIMPER) was also applied on FTIR absorption band group intensities to determine which band was responsible for group differences among soil types and depth classes. Bray-Curtis was used as distance measurement for ANOSIM, PERMANOVA and SIMPER. The proportional contribution of microbial, peat quality and other soil properties to variation in C mineralization were determined by Canonical correspondence analysis (CCA)-based variation partitioning analysis (VPA). The NMDS, ANOSIM, PERMANOVA, SIMPER and VPA analyses were carried out via the vegan package (R 3.1.2, R Development Core Team, 2014). When evaluating the effect of stratified mineral horizon and depth, peat and mineral samples were considered separately.

4.5 Results

4.5.1 Carbon mineralization and microbial abundance

Total (mg C-CO_2 kg^{-1} soil) and specific (mg C-CO_2 kg^{-1} SOC) C mineralization rates were determined from peat and mineral horizons in each of the five sub-types (Fig. 4.2). Material type had the greatest influence on cumulative total C mineralization. Cumulative C mineralization from peat samples was about 31-fold greater than that from mineral samples (p=0.00), but there were no differences between different types of mineral sediments (p=0.38) or among soil types (p=0.19). When only peat samples were considered, total C mineralization in surface peat was 4.1 times higher than in middle and deep peat (Table 4.3). Among sub-types, the only difference observed was lower C mineralization in middle peat from PMP1 and PMP2 (p<0.05). Cumulative specific C mineralization showed similar patterns. The exception was higher specific C mineralization in deep peat, rather than middle peat, from PMP1 and PMP2 compared to PP (p<0.05). Among depth classes, unlike PMP, PP cumulative specific mineralization in middle peat was higher than deep peat (p<0.01), which was negatively correlated with θv (p<0.05).

Microbial abundance (total PLFA concentration) was correlated (r = 0.67; p<0.01) with cumulative specific C mineralization by depth classes and among soil sub-types. When compared to surface peat, total PLFA concentration was about 50% and 80% lower in middle peat and deep peat, respectively (Table 4.3). Moreover, as with cumulative specific C mineralization, total PLFAs in middle peat were greater than that in deep peat in PP (p<0.05), but not in PMP. Among sub-types, similar to cumulative specific C mineralization, only middle peat had greater total PLFAs in PP than PMP (p<0.05).

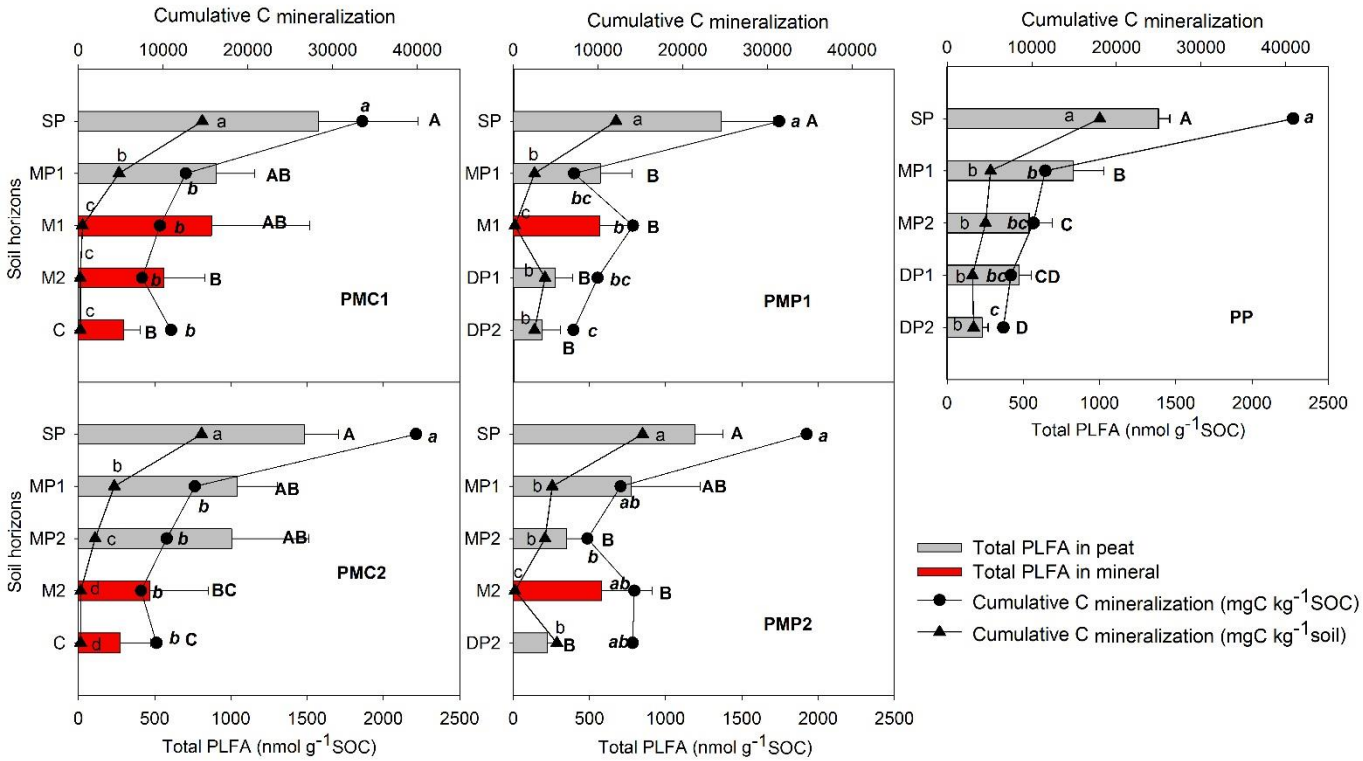

Fig. 4.2. Microbial abundance (total PLFAs) and cumulative (63 d) C mineralization in the different soil horizons of the five sub- types; SP: surface peat, MP: middle peat, DP: deep peat, M: silty mineral sediments, C: calcareous sediments. Tukey test was applied to each individual sub-type to test the differences of C mineralization and total PLFAs in different layers, $p<0.05$. Uppercase letters stand for difference levels of total PLFAs; lowercase letters for total cumulative C mineralization; bold and italic lowercase letters stand for specific cumulative C mineralization per kilogram of soil organic C.

Table 4.3. Significance levels from ANOVA of effects of sub- type and depth class on microbial abundant, total and specific C mineralization, and relative abundance of B:F, GP:GN bacteria and physiological stress biomarkers.

Factor	PLFA		Total C mineralization		Specific C mineralization		B:F		GP:GN		Stress	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Depth class	57.92	0.00	104.67	0.00	104.10	0.00	13.95	0.00	32.89	0.00	24.32	0.00
Sub-type†	3.45	0.01	2.29	0.07	1.44	0.23	2.00	0.11	2.31	0.07	3.17	0.02
Sub-type × Depth class	0.32	0.92	1.08	0.39	1.35	0.25	1.20	0.32	0.74	0.62	1.38	0.24

† Sub- type: Five sub- type are PMC1, PMC2, PMP1, PMP2, PP; PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

4.5.2 Microbial community structures

Stress biomarker (cy17:0/16:1w7c), B:F and GP:GN ratios varied greatly by depth class (Table 4.3): Stress biomarker and B:F were greatest in middle peat, while GP:GN were greater in middle peat and deep peat than in surface peat (Fig. 4.3). Among sub-types, differences in relative abundance of microbial groups were not consistent across depth class: in surface peat, PMC1 and PMC2 had the lowest B:F, GP:GN and Stress biomarker, followed by PP, PMP2, and then PMP1. In contrast, in middle peat and deep peat, only B:F was higher in PP than other sub-types (Fig. 4.3). Stress biomarker and GP:GN were positively correlated with θv, but were negatively correlated with TOC and both total and specific C mineralization rates (p<0.01).

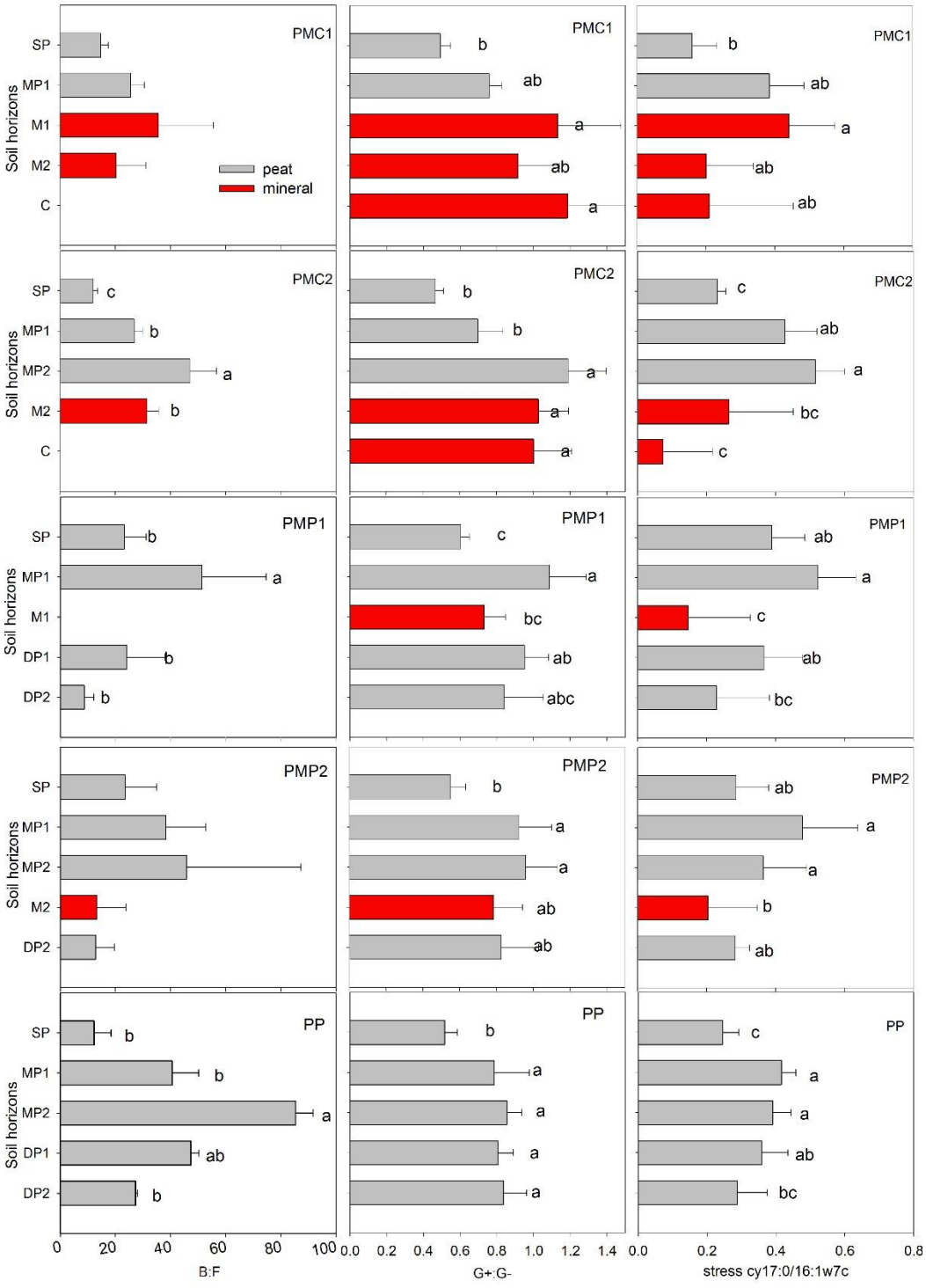

Fig. 4.3. Relative abundance of bacteria:fungi (B:F) (left), GP:GN bacteria (middle), and physiological stress biomarkers (right) in the different soil horizons of the five sub- types, PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat; SP: surface peat, MP: middle peat, DP: deep peat, M: silty mineral sediments; C: calcareous sediments.

Microbial community structure differed greatly between peat and mineral samples (NMDS ordination; data not shown), suggesting that material type was a key factor driving variability. When we excluded mineral samples, ANOSIM analysis suggest both depth classes and soil types affected microbial community structure (p<0.01), whereas depth classes (R=0.67) had greater effect than soil types (R=0.30). The NMDS analysis also showed that microbial community structures in peat samples were mainly determined according to the three depth classes rather than by soil type (Fig. 4.4). Stress biomarker was positively correlated with middle peat; C:N ratio was positively correlated with deep peat; and TN was positively correlated with surface peat (Fig. 4.4, p<0.10). The effect of soil types was mainly reflected in deep peat, which showed that communities in PP were distinct from those in PMP (ANOSIM R=0.41; Fig. 4.5). The environmental factors were correlated with microbial community distribution (p<0.15), where total PLFAs and θv were positively correlated with PP (Fig. 4.5).

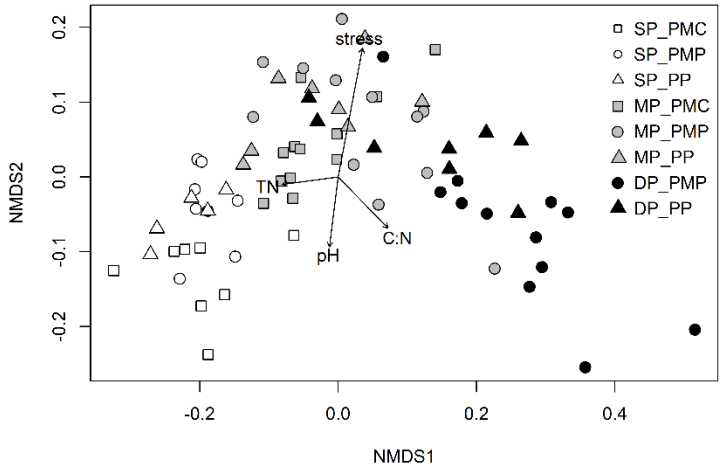

Fig. 4.4. Nonmetric multidimensional scaling (NMDS) analysis of microbial communities of peat samples from the three main depth class in the three main soil types (final stress = 0.103). PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat; SP: surface peat, MP: middle peat, DP: deep peat.

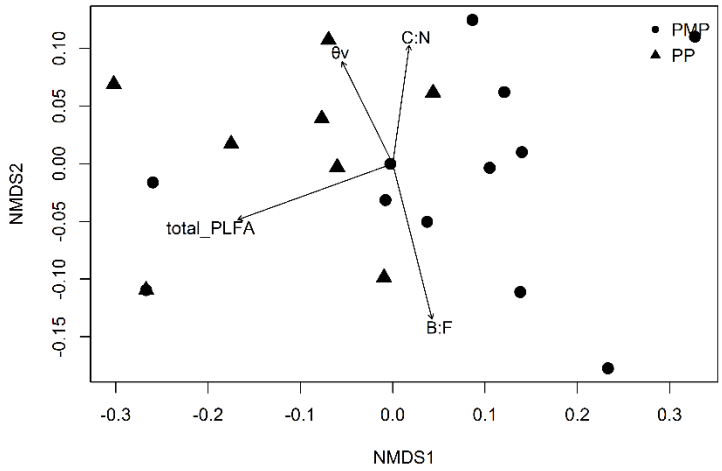

Fig. 4.5. Nonmetric multidimensional scaling (NMDS) analysis of microbial communities of peat samples from deep peat in main soil types (final stress = 0.157). PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

4.5.3 Peat C chemistry

The C functional groups in different depth classes from five sub-types were distinguished by FTIR spectra (Fig. 4.6). In all spectra, the highest intensity absorption bands were at 950-1170 cm^{-1} (average 1033 cm^{-1}). This absorption band is typical for polysaccharides, which suggested high labile C (LC) content in all peat samples. Other common absorption bands represented biochemically recalcitrant C fractions (RC), like phenolic and aliphatic, aromatic and lignin groups (~1414 cm^{-1}, ~1516 cm^{-1}, ~1630 cm^{-1}). By PERMANOVA, it was found that band group intensities mainly differed among different sub-types and tended to differ among depth classes (Table 4.4). The band ~1630 cm^{-1} had the greatest contribution (~53%) to group differences, which suggested different relative concentrations of lignins were the main differences in peat material. Among sub-types, PP showed the highest intensity of lignin, whereas PMC2 showed the lowest intensity, which represented the enrichment of lignin in PP. Intensities of recalcitrant C band group varied among depth classes in PMP1, PMP2 and PP, where the intensities of recalcitrant C band group were higher in middle peat than surface peat, but lower in deep peat (p=0.08). However, in PMC1 and PMC2, absorption band group did not change with depth classes (p=0.40).

Table 4.4. Significance levels from PERMANOVA of effects of sub-types and depth classes on absorption band group intensities.

	Sum of sqrs	**df**	**Mean square**	**F**	***p***
Depth class	0.144	2	0.036	0.658	0.131
Sub-types †	0.245	4	0.061	1.120	0.009
Sub-types × Depth class	-1.072	8	-0.067	-1.226	0.477

† Sub-types: Five sub-types are PMC1, PMC2, PMP1, PMP2, PP; PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

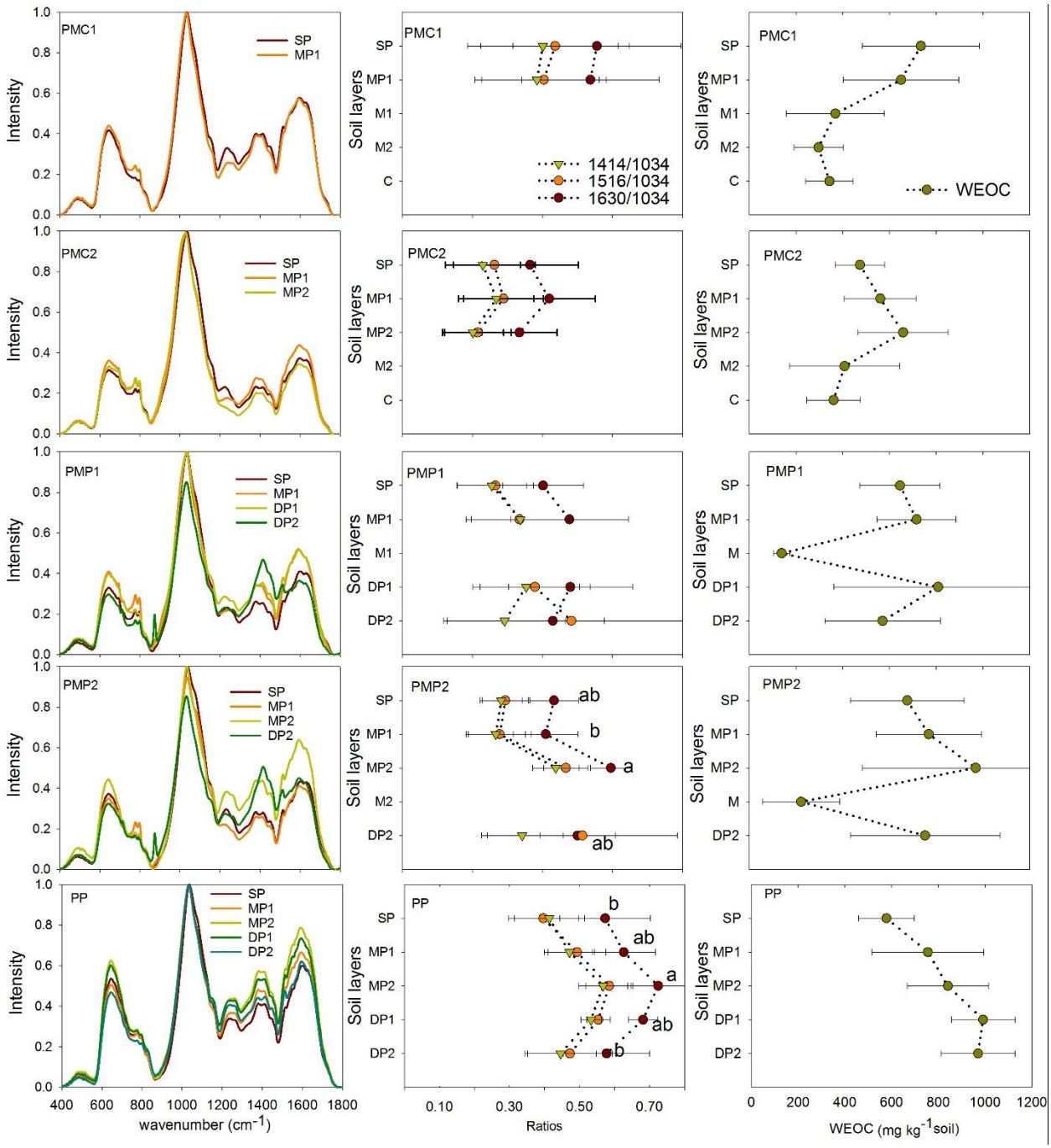

Fig. 4.6. FTIR spectra (normalized and averaged, n=4) (left), RC:LC (middle) and WEOC (right), of peat samples from different layers in sub- type. 1630/1034, 1516/1034 and 1414/1034 are the ratios of phenolic and aliphatic/polysaccharide, aromatic/polysaccharide and lignin/ polysaccharide.

The RC:LC ratios differed among sub-types (Table 4.5). PP had the highest ratios followed by PMC1, PMP2, and PMP1, while PMC2 had the lowest ratios. Within each sub-type, these ratios showed similar patterns, i.e., ratios increased from surface peat to middle peat and then decreased, indicating middle peat was more decomposed and enriched with more recalcitrant carbon (Fig. 4.6). However, only changes of 1516/1034 and 1414/1034 with depth classes were significant (p=0.027 and p=0.090), especially in PMP2 and PP.

Water extractable organic C was about 2.5 times greater in peat samples than mineral samples (p<0.001). In peat samples, WEOC showed significant differences among depth classes and sub-types (Table 4.5). Among soil sub-types, the highest WEOC content was found in PP, whereas PMC2 had the lowest content; the other three were at similar levels (Fig. 4.6). Among depth classes, WEOC content in peat samples increased from surface peat to middle peat, and remained the same from middle peat to deep peat for PMP and PP (Fig. 4.6).

Table 4.5. Significance levels from ANOVA of effects of sub- type and depth classes on the WEOC and RC:LC.

Factor	1414/1034		1516/1034		1630/1034		WEOC	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Depth class	2.10	0.09	2.90	0.03	1.48	0.22	2.86	0.03
Sub- type†	15.48	0.00	6.70	0.00	13.33	0.00	2.73	0.04
Sub- type × Depth class	0.90	0.53	1.31	0.25	0.86	0.56	1.03	0.42

† Sub- type: Five sub- type are PMC1, PMC2, PMP1, PMP2, PP; PMC: peat/silty mineral sediments/ calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

4.5.3 Contribution of different factors on variation in C mineralization

To quantify the contribution of microbial properties, substrate quality and other soil properties to changes in peat C mineralization with depth and among soil types, CCA-based variation partitioning analysis (VPA) was conducted. Microbial biomass, B:F and GP:GN were considered as microbial properties; C:N, RC:LC (1630:1034) and WEOC were selected as substrate quality; gravimetric water content, pH, NH_4^+-N and NO_3^--N content were used as other soil properties (Fig.4.7). Among these three factors, soil properties showed greatest contribution to variation of C mineralization (12.5%). Water content alone accounted for approximately 78% of the soil properties contribution. In addition, the interaction of substrate x microbial properties

x soil properties explained over 40% of the C mineralization variation. This indicated that the three factors mainly interacted with each other to affect C mineralization.

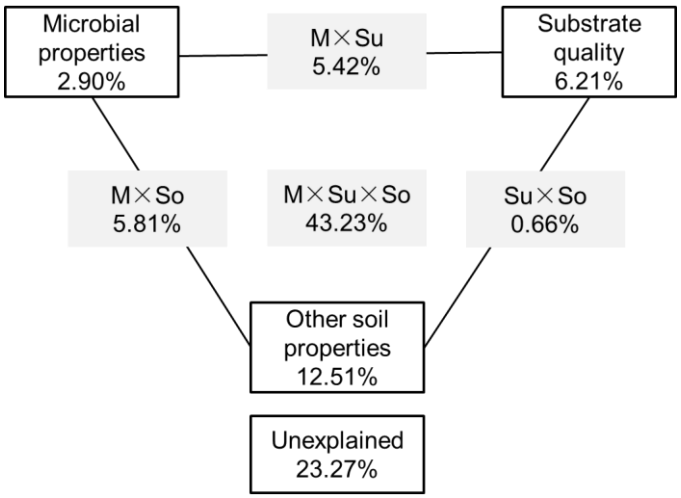

Fig.4.7. CCA-based variation partitioning analysis (VPA) shows the contribution of microbial properties (M), substrate quality (Su) and soil properties (So) to variation in C mineralization.

4.6 Discussion

Depth had a greater effect on C mineralization, microbial abundance and community structure than did stratigraphy (Table 4.3). However, the changes of microbial abundance and specific C mineralization were complicated by the presence of mineral horizons (Fig. 4.2 and Table 4.3), as discussed below. Microbial abundance and C mineralization rates were within the range reported by others (Ranneklev and Bååth, 2003; Updegraff et al., 1995). Consistent with other peatland studies, microbial abundance and C mineralization decreased and microbial community structures changed considerably with depth in peat profiles lacking a mineral horizon (Fig. 4.2), likely in response to declining temperature, oxygen availability and nutrient content at depth (Andersen et al., 2013; Basiliko et al., 2007; Davidson and Janssens, 2006; Lüdemann et al., 2000).

4.6.1 Impact of stratified mineral horizons on surface peat

Whether and how stratified mineral horizons affected C mineralization, microbial abundance and community structure, and peat C chemistry depended on depth. In surface peat, there was a minor influence of stratified mineral horizons. This is because surface peat did not directly connect with mineral sediment and the C source is mainly from aboveground vegetation.

The only measured difference among the soil types was in microbial community structure. For example, B:F, GP:GN and physiological stress biomarker were higher in PMP and PP than PMC, as there was greater abundance of recalcitrant C derived from willows and mosses, which were the dominant vegetation in PMP and PP (Biasi et al., 2005; Grogan and Cronan, 1997).

4.6.2 Impact of geomorphic history on middle peat

The presence of a stratified mineral horizon and changes of peat origin indicate different geomorphic history that affected middle peat characteristics (i.e., peat formed on top of mineral sediments in PMP and PMC). The highest RC:LC was found in middle peat across soil types in this study. A possible explanation is that the paleo-climate became drier (Vance et al., 1995) during middle peat formation (about 6880-4110 years B.P.) (Janzen and Westbrook, 2011), which should enhance decomposition. During the initial soil survey we noted that at around 25-50 cm (middle peat) in PP, at similar depths to where middle peat or mineral sediment accumulated in PMC and PMP, the dominant peat forming plants transitioned from *Sphagnum* moss to sedge. This change of dominant vegetation indicates a shift of climate from humid, *Sphagnum*-forming conditions to relatively drier, sedge-forming conditions (Gunnarsson et al., 2002). Drier conditions would have facilitated decomposition – and slowed peat accumulation – during middle peat formation, leading to a more decayed layer with higher RC: LC (Broder et al., 2012; Malmer et al., 2005). In addition, the highest RC:LC ratio in middle peat suggested poor substrate quality that could result in the starvation of certain GN and increasing physiological stress (Bååth and Anderson, 2003; Wixon and Balser, 2013).

The chemical influence of mineral sediments may also have promoted decomposition in middle peat of PMC and PMP during an earlier period in the peatland's geomorphic history. For example, mineralization (mg C-CO_2 kg^{-1} soil) differed among soil types: PP showed higher total C mineralization than PMP (over 34.4%) or PMC (over 32.6%) (Fig. 4.2). For PMC, the presence of silty and calcareous mineral horizons contributes to more nutrients and higher pH (Table 4.1; Wang et al., 2016). Consequently, the decomposition rate would have been greater at the time of peat formation (Hodgkins et al., 2014), resulting in lower WEOC (p=0.029) and higher RC:LC ratios. For PMP, even with its lower pH and lack of calcareous sediments, the interaction of peat and silty mineral horizons might also have promoted decomposition during peat formation by providing ions and electron acceptors (Biester et al., 2003; Broder et al., 2012).

Therefore, geomorphic history affected substrate quality (WEOC and RC:LC) and other soil properties (pH and nutrient content) which in turn affected C mineralization rate in this study (Fig.4.7.).

In addition, stratified mineral sediments may provide physicochemical protection that affected historical C susceptibility to decomposition and continues to affect present-day specific C mineralization. Middle peat in PP had similar specific C mineralization to PMP and PMC (Fig. 4.2) when scaled by the amount of OC present (mg C-CO_2 kg^{-1} SOC), although with higher RC: LC (Fig. 4.6), C mineralization in PP was expected to be lower. This suggests that C susceptibility to decomposition in all three soil types is similar despite different substrate qualities. It is possible that mineral sediments increased C stability in middle peat of PMC and PMP. The RC:LC ratios only reflected one aspect of organic matter stabilization, i.e., biochemical stabilization; however, interaction with minerals also enhances organic matter stabilization (Artz et al., 2008; Krull et al., 2003). The silty mineral sediments in PMC and PMP were mixed with peat material in the transition zone, which might provide physico-chemical protection for OM, especially for aromatic C (Han et al., 2016). It has been discussed that biochemically recalcitrant C, such as that found in PP, could be vulnerable to decomposition when lacking physicochemical protection (Lehmann and Kleber, 2015). Therefore, PP had similar specific C mineralization rates to PMC and PMP . In sum, the stratified mineral horizon affected C mineralization via increasing pH, providing electron acceptors and physicochemical protection in the peatland's geomorphic history. However, mineral sediment may also affect present-day C mineralization by affecting groundwater hydraulic conductivity.

4.6.3 Impact of stratified mineral horizon on middle peat and deep peat due to hydrological conditions

Stratified mineral horizons can also affect subsurface peat via influencing hydrological conditions. In a previous study, it was found that the mineral horizon impedes water infiltration because the interbedded silty mineral lens had lower groundwater hydraulic conductivity (Janzen and Westbrook, 2011). This would lead to higher water content above the mineral sediment (Wang et al., 2016b) and cause episodic anaerobic conditions, which not only slow C mineralization rates, but also influence the by-products of metabolism, resulting in less degradable OC (Davidson and Janssens, 2006) and a shift of microbial community structures to

more abundance of oligtrophs (Prosser et al., 2007) as supported by middle peat RC:LC ratios, as well as GP:GN ratios and stress biomarker. The anaerobic conditions caused by higher water content altered microbial community structures and interacted with poor substrate quality, which then slowed down decomposition in middle peat and resulted in similar mineralization rates in middle and deep peat (Fig.4.7, Davidson and Janssens, 2006).

At the same time, hydrologic conditions not only affected middle peat, but also deep peat, resulting in greater specific C mineralization in deep peat of PMP than that of PP. Since the interbedded silty mineral lens slows water infiltration and organic acid transfer from middle peat to deep peat in PMP (Janzen and Westbrook, 2011), deep peat in PMP was drier and less acidic compared with PP (Wang et al., 2016b). When deep peat was incubated in surface-like conditions (22°C, field moisture), θv in PMP was lower and encouraged C mineralization, as lower θv represents more aerobic conditions in peatland, which is a key regulator for C decomposition (McLatchey and Reddy, 1998). This explains the higher specific C mineralization in PMP and its negative association with θv (p<0.10). On the other hand, without mineral sediment, a large amount of water and organic acid can leach into the deeper layers of PP. This would result in a C mineralization rate that is limited by more anaerobic and acidic conditions, in spite of the deep peat of PP having higher WEOC (p<0.05) and microbial biomass (p=0.07).

Hydrologic conditions also affected microbial community structures in middle peat and deep peat. Middle peat showed the greatest indication of B:F and stress biomarker in microbial PLFA profiles, and the microbial community structure was positively correlated with stress biomarker. These results suggest environmental stressors impacted microbial community structure. In addition to the poor substrate quality in middle peat as discussed before, another possible explanation for the higher B:F and physiological stress is that the higher θv (Wang et al., 2016b) above the mineral sediments created anaerobic conditions (Bååth and Anderson, 2003; Wixon and Balser, 2013). Compared with fungi, bacteria are also more abundant in and easily adapted to stressful conditions like anaerobic, acidic and poor substrates (Andersen et al., 2013). However, middle peat was not more acidic than deep peat.

The microbial community structure in deep peat was also influenced by differences in hydrologic conditions with the presence of stratified mineral horizons. The analysis shows that the structure in PMP was different from that in PP (ANOSIM R=0.41, p<0.01): microbial

community structure was positively correlated with θv and microbial biomass (Fig. 4.5). Since deep peat in PMP is drier than that in PP (Wang et al., 2016b), the redox potential may have been different, which is an important factor regulating microbial community structure (Bossio and Scow, 1998). Meanwhile, with slower infiltration, less organic acid and WEOC would be expected to leach into deep peat in PMP, resulting in higher pH and lower microbial biomass. As B:F is negatively correlated with pH at near-neutral conditions, the fact that B:F was negatively correlated with microbial community structures in PP was not surprising (Morris and Blackwood, 2007; Rousk et al., 2009).

4.7 Conclusion

The presence of mineral horizons had both physical and chemical influences on the C mineralization, substrate quality, and microbial community structure, and did so mainly at depth. Physically, the presence of a stratified mineral horizon affected hydrological conditions by leading to higher water content above the mineral horizon and drier conditions below the mineral horizon. This inhibited C mineralization in middle peat and encouraged C mineralization in deep peat in PMP to the point where the rate was similar to that in middle peat and deep peat. Chemically, mineral horizons affected C mineralization and substrate quality by increasing pH and providing electron acceptors during peatland's geomorphic history in middle peat. In addition, the physico-chemical protection of C from mineral sediment further restricted C mineralization in PMP and PMC. The relative dominance of these controls may have varied throughout the geomorphic history of the peatland. At present, the conditions have led to effects on the microbial community structure, i.e., highest B:F and stress biomarker in middle peat and different microbial community structures in deep peat with and without mineral sediments. Besides the effect of mineral horizon, depth had an even greater effect: C mineralization and microbial abundance decreased considerably with depth, and microbial community structure changed significantly. Our findings indicate stratified mineral horizon have an important impact on peatland biogeochemistry at this mountain site, and raises the question of how widespread and important this influence would be. Importantly, these effects were restricted to subsurface peat. When estimating global C stocks under a changing climate, our results indicate it is necessary to carefully consider C mineralization processes occurring below the peat surface, especially for peatlands that may have mineral sediment interbedding.

5. Responses of A Mountain Peatland to Changing Climate: A Microcosm Study of GHG Emission and Microbial Community Dynamics

5.1 Preface

The previous two chapters demonstrated that stratified mineral sediments affected the spatial distribution of soil properties, substrate quality and C&N cycling rates. These factors are important when evaluating greenhouse gas (GHG) emissions. Therefore, it is likely that mineral sediments in peat profiles may affect GHG emissions and/or peatland feedback to global warming. As microorganisms are important mediators of GHG production, it is necessary to take microbial community dynamics into consideration. To study the GHG emission and microbial community structure changes from soil profiles with and without a mineral horizon under climate warming conditions, I conducted a microcosm study, incubating two soil types under different temperature-water table treatments. This chapter is not yet published.

5.2 Abstract

Peatlands in western Canada are carbon (C) and nitrogen (N) sinks. However, the feedback of peatland C cycling to global warming is still uncertain, especially for subsurface peat. In the northern Rocky Mountains, peat stratigraphy can be complex: peat deposits range from thick to very thin, and can be interrupted by mineral sediment layers. It is possible that the interaction of peat and mineral layers may make the feedback of greenhouse gas (GHG) emissions to global warming more complex. In addition, microbial communities are the main mediators of C and N cycling. To study the effect of stratified mineral sediments on GHG emissions, how GHG from peat might respond in a changing climate, and whether mineral horizons affect GHG emissions by influencing microbial community structure, I conducted a microcosm experiment.

Two soil types – sedge peat underlain by mineral/calcareous sediments (PMC) and sedge peat underlain by moss peat (PP) – were incubated for 28 days under four treatments: current temperature/current water table, higher temperature/current water table, current temperature/lower water table, and higher temperature/lower water table. Surface GHG emissions and GHG concentrations from four depths (surface, above water table, below water table and above mineral contact) were monitored. Following incubation, peat cores were disassembled and measured for GHG production rates (four depths), apparent enzyme activation energy (E_a), and bacterial community structure (above and below the water table); the same measurements were completed on cores that had not been incubated. Results indicated that high temperature increased GHG emission (CO_2 by 28%, CH_4 by 133% and 178% N_2O) and concentrations – at surface and at depth (CO_2 by 32 ~ 83%, CH_4 by 200 ~ 1600% and -61 ~ 230% N_2O) – in most samples. Both CO_2 and N_2O from subsurface were higher in PP than PMC. In addition, microbial community structures were mainly grouped according to soil type. Moreover, there was an interaction effect of temperature and soil types for N_2O: concentration and production rates in PP were more increased by high temperature. This was possibly because of more labile C and lower pH in PP and the increased E_a for N_2O generation. Overall, our findings suggest that peat profile with mineral horizons tend to produce less GHG and may moderate N_2O production under a warming climate.

5.3 Introduction

Boreal and subarctic peatlands store about 30% of global soil carbon (C) and account for 4-10% of global CH_4 emissions (Gorham, 1991; Mikaloff Fletcher et al., 2004). Peatlands in western Canada are a large C pool, as well as a long-term sink for nitrogen (N) (Moore et al., 2004). However, peatlands can also be a substantial source of greenhouse gases (GHG) under global warming (Roulet, 2000). It has been demonstrated that CO_2, CH_4 and N_2O production are all elevated by higher temperature (Ambus et al., 2006; Dorrepaal et al., 2009; Treat et al., 2014; Wunderlich and Borken, 2012). However, hydrologic changes, such as a lower water table, are more likely to increase CO_2 emission but decrease CH_4 emission, as CH_4 production requires strictly anaerobic conditions (Holmes et al., 2014). The highest N_2O production is most likely to appear in variable aerobic-anaerobic conditions, which are common under fluctuating water table conditions (Hefting et al., 2004).

In addition to environmental factors, substrate quality is also important for regulating GHG emissions (Wright et al., 2011). Labile C forms, such as polysaccharides, are readily decomposed via C and N cycling and are most vulnerable to environmental change, especially increasing temperature (Preston et al., 2006). It has been found that CH_4 emissions are more stimulated by temperature when labile C forms are abundant (Wright et al., 2011). Previous studies also observed that peatland C availability has a greater influence on N_2O fluxes than temperature (Danevčič et al., 2010; Klemedtsson et al., 2005).

Carbon and N cycles are largely mediated by microorganisms; the temperature control on GHG emissions is mainly via its effects on microbial activity and/or microbial biomass and communities. Microbial communities can affect soil functions at the phyla, class and even lower taxonomic levels (Banerjee et al., 2016; Fierer et al., 2007). In addition, temperature dependence of microbial activity can be reflected in apparent enzyme activation energy (E_a) (Yavitt et al., 2000), and the E_a of a reaction is regulated by substrate quality and microbial community structures. For example, decomposition of recalcitrant materials requires more energy than labile materials, and therefore has higher E_a (Davidson and Janssens, 2006). Different microbial community structures produce different extracellular enzymes, which results in different E_a (Davidson and Janssens, 2006; Sinsabaugh, 1994). In addition, substrate quality can affect microbial community structures. For example, ecologically, microbes can be classified into two

groups: copiotrophs (*r*- strategists) and oligotrophs (*k*- strategists) (Fierer et al., 2007). Copiotrophs are usually more abundant in nutrient rich conditions and grow faster than oligotrophs, whereas oligotrophs prefer nutrient poor conditions (MacArthur and Wilson, 1967). For GHG production, it has been found that E_a and microbial community structures change with changing temperature, hydrology interactions and substrate quality (Inglett et al., 2012; Lloyd and Taylor, 1994; Yavitt et al., 2000).

In the peatlands of the northern Rocky Mountains, peat usually develops with complex stratigraphy, including shallow underlying and interbedded mineral sediments (Margalef et al., 2013). Previous studies found that peat profiles with and without mineral sediments significantly vary in terms of key soil properties, substrate quality and C and N cycling rates (Wang et al., 2016a; Wang et al., 2016b). This suggests that the interaction of peat and mineral layers may make the feedback of greenhouse gas (GHG) emission to global warming more complex in subsurface layers. Therefore, the objectives were: 1) to study the effect of peat and mineral sediment layering on GHG emissions and how these materials might respond in a changing climate; and 2) to investigate whether changes were due to changes in biogeochemical processes rates, apparent enzyme activation energy or microbial community structure.

5.4 Material and Methods

5.4.1 Site description and soil collection

Sibbald Creek research wetland is a hummocky mountain peatland within a relatively level valley basin in the Kananaskis region of southern Alberta, Canada (Latitude: 51.06N, Longitude: 114.87W) (Fig. 5.1A). The mean air temperature is 14.5°C for July and mean average precipitation is 653 mm (Janzen and Westbrook (2011); average water table depth during summer is 22.9-26.4 cm below surface (based on data collected from 2006 to 2009). Three distinct soil types were identified in the peatland: sedge peat/silty mineral/calcareous sediment (PMC) in the southwest zone of the basin; sedge peat/moss peat profiles (PP) in the northeast of the basin; and sedge peat/silty mineral/moss peat (PMP) in the middle (Fig. 5.1B) as described in Wang et al. (2016a; Chapter 3). Only PP and PMC were sampled in this study, because in previous work, these soil types showed the most significant differences in terms of soil properties and C and N mineralization and nitrification. Basic soil properties were described in Wang et al. (2016b) and are summarized in Table 5.1. Samples were collected to just above the

mineral horizon in PMC or equivalent depth in PP, as no significant difference was found between middle peat and deep peat. Sedges (predominately *Carex aquatilis*) are the most common vegetation in PMC; whereas sedges and willows (*Salix spp.*) are co-dominant in PP.

Six representative sampling points were selected from each soil type (PMC and PP). At each sampling point, five intact soil cores were taken from the surface to approximately 50 cm depth (the average depth to mineral sediments) by a hand corer (ID=9.3cm). Living plants were removed and cores were assembled into PVC tubes in the field. Each PVC tube (OD = 10.7 cm, ID = 9.9 cm, length = 60 cm) was capped on the bottom and pre-drilled with 40 4-mm holes. The tubes were 10 cm longer than the collected soil cores: at the top, 5 cm were used as headspace during gas sampling (Jungkunst et al., 2008); 5 cm at the base was reserved and used for repacking mineral sediments or peat to mimic mineral and peat interaction in the field (Fig. 5.1E). The mineral sediments were taken by Dutch auger and repacked into the PVC tubes in the lab according to their bulk densities. The cores were preserved in coolers during transport back to the lab and then stored at -20°C until use. The PVC tubes were wrapped with a permeable screen mesh (0.84 mm) to allow water movement without losing peat and incubated at 4°C for one week before initiating the microcosm experiment. Of the five intact cores, four were subjected to temperature - water table treatment; the fifth served as a Background core.

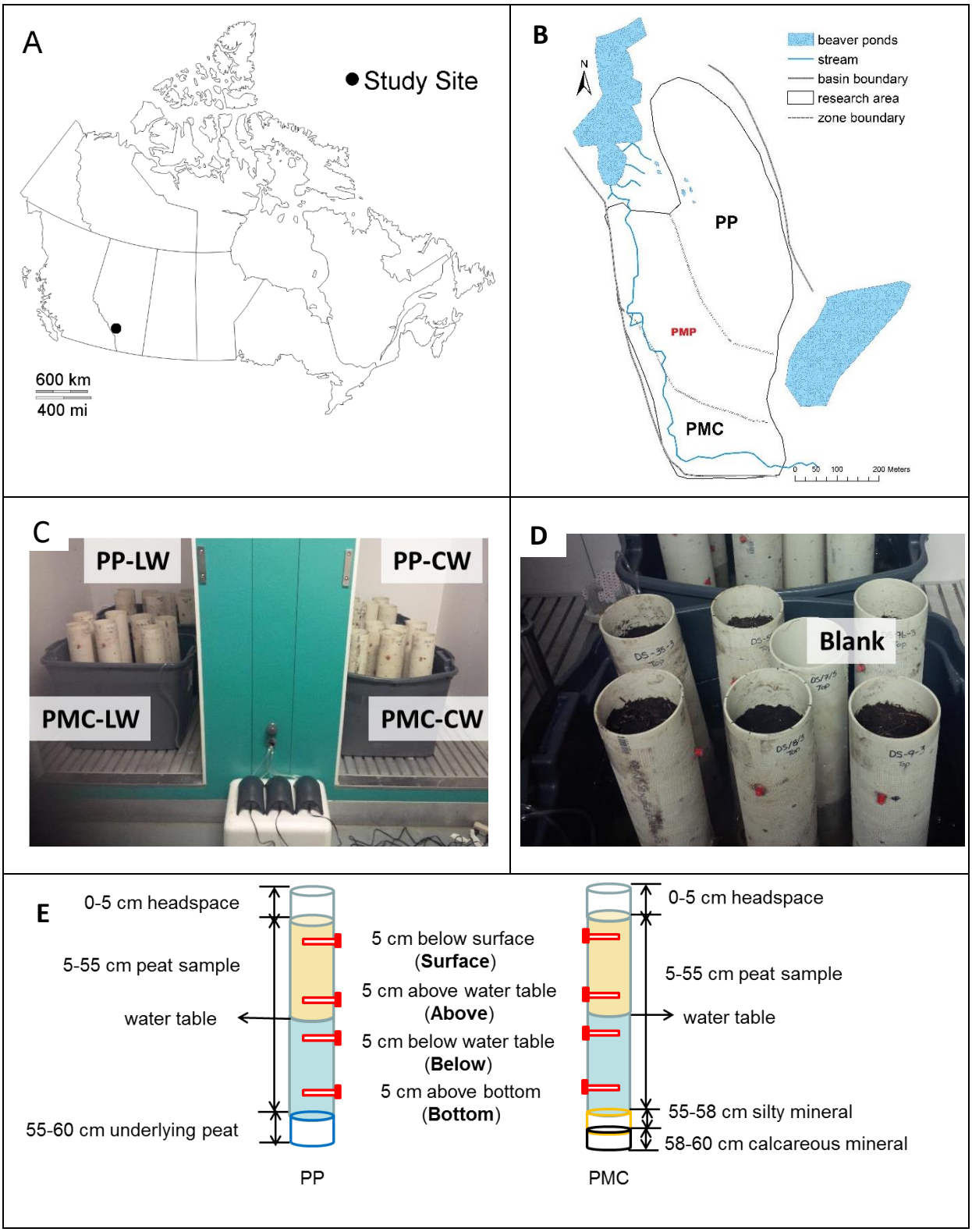

Fig. 5.1. A) Location of the field site. B) Research area and zones according to stratified mineral horizons. PMC and PP were selected zones in this study. C) Incubation treatment setup under one temperature treatment. D) Six replicates and one blank core in one treatment. E) Soil core assembling description and demonstration of four sampling ports at different depths.

Table 5.1. Soil characteristics of the two soil types in this study: peat/silty mineral/calcareous sediment (PMC) and sedge peat/moss peat (PP).

Soil type	Horizon	Depth (cm)	Fiber content	Von Post	Parent materials	Munsell color	θv (cm^3 cm^{-3})	pH	TOC § (%)	TN ¶ (%)	C:N	WEOC ‡ (mg kg^{-1})
PMC (Limnic Humisol) †	Om1	0-10	30%	5	Peat (Sedges)	10YR2/2	0.52±0.07	6.24±0.32	36.45±4.87	2.62±0.36	13.98±1.27	473.6±105.1
	Om2	10-20	25%	3 or 4	Peat	10YR2/2	0.75±0.05	5.78±0.19	32.73±8.33	2.52±0.56	12.93±0.64	560.4±153.9
	Oh	20-40	15%	5	Peat	10YR3/3	0.80±0.08	6.05±0.16	22.51±7.80	1.71±0.54	12.75±1.07	658.0±192.7
	Bg	40-60			Silts	10YR5/6						
	Ck	60-70+			Marl							
PP (Typic Mesisol)	Om1	0-10	30%	5	Peat (Sedges)	10YR2/2	0.55±0.07	6.04±0.41	43.10±0.99	2.66±0.27	16.34±1.63	578.0±118.9
	Om2	10-20	30%	5	Peat	10YR3/4	0.72±0.13	5.68±0.27	44.55±2.30	3.14±0.18	14.20±0.94	754.9±239.1
	IIOf	20-55+	65%	4	Peat (Moss & Sedges)	10YR4/6	0.64±0.11	5.62±0.17	42.74±2.04	2.90±0.31	14.83±1.25	840.7±174.0

† According to Canadian System of Soil Classification (Soil Classification Working Group, 1998).
‡ WEOC: water extractable organic carbon.
§ TOC: total organic carbon.
¶ TN: total nitrogen.

5.4.2 Microcosm experiment set up

To study GHG emissions, a controlled temperature and water table level microcosm experiment was conducted. Experimental conditions, summarized in Table 5.2, were determined based on published global warming scenarios – an estimated water table decline in peatlands of 7.1 to 14.4 cm is expected under climate warming (Roulet et al., 1992), commensurate with a 2 to 3°C increase in temperature in during the summer growing season in central North America (IPCC, 2007). The high temperature treatment of 25°C was set to evaluate the maximum potential changes (i.e., maximum daily temperature in field during summer = 22°C plus 3°C increase under climate warming condition). The low water table treatment was to lower the average water table by 15 cm. For each treatment, cores from each of the six sampling points in each soil type were used as replicates (n = 6). Soil cores were put into eight (4 treatments × 2 soil types) open top rectangular tanks (45.7 cm L×30.5 cm W×61.0 cm H) with gravel in the bottom. The water used in the microcosm experiment was adjusted to the same pH and ion concentration as the average of water samples from both East Inlet and Bateman Creek (Table B.1). Water table was set according to Table 5.2 and monitored every other day. Distilled water was added when necessary to maintain the water table level in the water tanks. In each tank, a blank core (a PVC tube with no soil) was used for base reading of GHG fluxes and concentration.

Table 5.2. Environmental conditions for microcosm experiment.

Temperature	**Water table**
Current temp. † (CT) 15° C	Current water table ‡ (CW) 25 cm below surface
Current temp. (CT) 15° C	Low water table ¶ (LW) 40 cm below surface
High temp. § (HT) 25° C	Current water table (CW) 25 cm below surface
High temp. (HT) 25° C	Low water table (LW) 40 cm below surface

† Current temperature: average daily air temperature during summer in the field.
‡ Current water table: average water table during summer in the field.
§ High temperature: maximal average daily air temperature could achieve under climate warming scenario during summer in the field.
¶ Low water table: average water table under climate warming scenario during summer in the field.

5.4.3 Gas measurements

Net GHG emissions from the soil column were measured on day 1, 3, 5, 7, 14, 21 and 28, by taking 20 mL gas samples from the headspace (~5 cm length × 10.3 ID) of each core by

syringe, after sealing the PVC core with a flexible PVC cap (ID = 10.7 cm) for 30 min (Wright et al., 2011). The net GHG emission was calculated as the differences in gas concentration between the soil cores and the blank cores divided by time (i.e., 30 min). At the same time, concentrations of CO_2, CH_4 and N_2O were measured from four depths (Fig. 5.1E). At 5 cm below surface (Surface) and 5 cm above water table (Above), 5 mL soil air samples were collected by syringe through septum sampling ports and injected into 12 mL evacuated Exetainers (LabCo Inc., High Wycombe, UK) with 15 mL pure N_2. At 5 cm below the water table (Below) and 5 cm above bottom (Bottom), because of high GHG concentration, only 3 mL of soil solution was sampled in the same way. Then the Exetainer with 3 mL soil solution and 15 mL pure N_2 was shaken to release GHG and 3 mL gas from headspace was transferred into a secondary Exetainer with 17 mL pure N_2. After equilibrating at 25°C, the concentration of CO_2, CH_4 and N_2O in the headspace were determined by a gas chromatograph (Bruker 450 GC, Bruker Biosciences Corporation, USA). Cumulative GHG emission was calculated by calculating the area under the curve over incubation time. Specifically, thermal conductivity detector (TCD) was used for CO_2; flame ionizer detector (FID) was used for CH_4; and electron capture detector (ECD) was used for N_2O. Then data was processed via Varian MS Workstation (version 6.9.3). The GHG concentrations in soil air and water at each depth were calculated according to (Fiedler et al., 2005).

5.4.4 Biogeochemical processes rates

After 28 d of incubation, soil cores (treated and Background) were disassembled and separated into four segments: 0-5 cm from surface (Surface), water table to 5 cm above water table (Above), water table to 5 cm below water table (Below), and bottom to 5 cm above bottom (Bottom). Each segment was carefully mixed and divided into five sub-samples: one subsample (~100 g) was repacked into 1 L Mason jar according to bulk density, two subsamples (~100 g each) were repacked into plastic containers with caps, one subsample (~10 g) was preserved in sterilized wrap bags and stored in -20°C for molecular analysis, and the remaining one (~10 g) was used to measure water content, $[NO_3^-]$ and $[NH_4^+]$.

Samples in Mason jars were flushed with lab air and incubated at room temperature for 24 h. After 24 h incubation, 20 mL gas samples were taken from the jar headspace and subjected to GC measurements for CO_2, CH_4 and N_2O concentrations. Gas concentrations were converted

into GHG production rates based on incubation duration (24 h) and mass of subsamples. Carbon dioxide production rates represented net C mineralization during 24 h.

The two subsamples in plastic containers were labeled with 6 mL (0.8 mL×8 time) $K^{15}NO_3$ (15 μg N mL^{-1} at 98 atom %). Within 20 min of injection with $K^{15}NO_3$, subsamples from one plastic container were homogenized and sampled to determine $^{15}NO_3$ at time 0. After 24 h, samples in the remaining tub were used to determine $^{15}NO_3$ at time 24. Before and after the 24 h incubation, ^{15}N content was determined by the modified diffusion procedure (Bedard-Haughn et al., 2004). Gross nitrification rates (mg N kg^{-1} soil d^{-1}) were determined by calculating the changes in N concentration and ^{15}N content between 0 and 24 h according to equation 5.1 (Hart et al., 1994):

$$\text{Gross nitrification} = \frac{[NO_3^-]_0 - [NO_3^-]_t}{t} \times \frac{\log \frac{APE_0}{APE_T}}{\log \frac{[NO_3^-]_0}{[NO_3^-]_t}} \quad [5.1]$$

where t is the time (day), and APE is the atom % ^{15}N excess.

5.4.5 Apparent enzyme activation energy

Apparent enzyme activation energies for the production of CO_2, CH_4 and N_2O, and consumption of CH_4 and N_2O were determined by incubating peat under anoxic and oxic conditions, respectively, and monitoring headspace gas concentration over time at different temperature levels. High temperature treated cores (HT) and not treated (Background) cores from Above and Below in two soil types were used; peat right above or below water table was expected to be more sensitive to changing climate. The temperature levels, redox conditions and gas concentrations used in the process measurements are listed in Table 5.3, and were set according to method described in Yavitt et al. (2000), with spike concentrations adjusted. All samples were incubated for 24 h. After 24 h, 1 mL CH_4 or 5 mL N_2O were extracted from the headspace and injected into evacuated 12 mL Exetainer vials with 15 mL N_2. The concentration of CO_2, CH_4 and N_2O were measured by GC (Bruker 450 GC, Bruker Biosciences Corporation, USA).

The E_a (kJ mol^{-1}) for GHG generation and consumption were calculated according to the linear relationship of ln(k) to 1/T (Holtan-Hartwig et al., 2002):

$$\ln(k) = \frac{-Ea}{R}\frac{1}{T} + \ln(A) \quad [5.2]$$

where k is the process rate (mol kg^{-1} s^{-1}), T is the temperature (K), R is the gas constant (0.008314 kJ K^{-1} mol^{-1}), and A is the Arrhenius constant (mol kg^{-1} s^{-1}).

Table 5.3. Temperature levels, redox condition and spike concentrations for each biogeochemical processes.

Processes	Temperature	Redox condition	Spike concentration
CH_4 production	4°C	Anoxic (N_2)	CH_4 100 ppm, CO_2 233 ppm
	10°C	Anoxic (N_2)	CH_4 100 ppm, CO_2 233 ppm
	22°C	Anoxic (N_2)	CH_4 100 ppm, CO_2 233 ppm
CH_4 consumption	4°C	Oxic (lab air)	CH_4 100 ppm, CO_2 233 ppm
	10°C	Oxic (lab air)	CH_4 100 ppm, CO_2 233 ppm
	22°C	Oxic (lab air)	CH_4 100 ppm, CO_2 233 ppm
N_2O and CO_2 production	4°C	Oxic (lab air)	N_2O 5 ppm
	10°C	Oxic (lab air)	N_2O 5 ppm
	22°C	Oxic (lab air)	N_2O 5 ppm
N_2O consumption	4°C	Anoxic (N_2)	N_2O 5 ppm
	10°C	Anoxic (N_2)	N_2O 5 ppm
	22°C	Anoxic (N_2)	N_2O 5 ppm

5.4.6 16S rRNA sequencing

As for E_a, samples from high temperature treated and Background cores from Above and Below in two soil types were used in this evaluation. Peat materials were homogenized under sterile conditions and approximately 0.5 g was taken from each sample for genomic DNA extraction using the FastDNA SPIN Kit from MP Biomedicals (Santa Ana, CA, USA) following the manufacturer's standard operation protocol. Bacterial universal primer 515f/806r was used to amplify the 16S rRNA as follows: 94°C for 3 min, 28 cycles of 94°C for 30 s; 53°C for 40 s and 72°C for 1 min; 72°C for 5 min. Amplified samples were sent to McGill University and Génome Québec Innovation Centre for sequencing by Illumina® MiSeq (Illumina, San Diego, CA, USA). The sequencing data was trimmed via FASTQ Quality Trimmer by moving windows with a window length of 4, a step size of 1 and a quality threshold of 25. Then the trimmed sequences were analysis by Mothur software package (Schloss et al., 2009). Briefly, the barcodes and primers were removed from sequences. Then any sequence with ambiguous base call and/or longer than 310bp was removed. The SILVA database was used to align the sequences in Mothur (Pruesse et al., 2007). Sequences only appearing twice in the entire dataset were removed by

function of split.abund with cutoff = 2. Then chimeras were removed by function of chimera.uchime. 16S rRNA reference (RDP) was used to assign taxonomy. Operational taxonomic units (OTUs) were clustered at 3% divergence (97% similarity) then classified by Mothur. Across all 48 samples, 3151510 sequences and 9766 operational taxonomic units (OTUs) were identified. Subsampling (16438 sequences, the lowest) was used to calculate alpha diversity (Chao, Shannon and Simpson indexes) in Mothur.

5.4.7 Statistical analysis

Homogeneity of variances was tested by the Bartlett test. Normal distribution was tested by Shapiro-Wilk test and histogram. All GHG, biogeochemical production rates and E_a data were not homogenously or normally distributed, so the Generalized Least Square approach was used to fit the model. There are several ways to adjust the residual variance structures. To choose the right variance structure, "varFixed", "varPower", "varExp" and "varConstPower" functions were tested and the model with the lowest AIC was selected. As different parameters had different residual variance structures, different methods were used for adjustment. For example, varPower (residue variance structures adjusted according to exponential of covariate) was used for E_a for N_2O production data; while varExp (residue variance structures adjusted according to power of covariate) was used for CH_4 concentration (Zuur et al., 2009). The ANOVA (anova function in R 3.1.2 package, R Development Core Team, 2014) was used to test the differences among treatments and between soil types. Relative abundance of bacterial community groups was normally distributed and were tested by ANOVA by fitting general linear model for differences among treatments and between soil types. Non-metric multidimensional scaling (NMDS) was used to describe bacterial community structure distribution via the PC-ORD statistical package, Version 6 (McCune and Mefford, 1999), Sorensen method was used for distance measurement.

5.5 Results

5.5.1 GHG emission and concentration by depth

Cumulative CO_2 emissions (Fig. 5.2A) were higher in PP than in PMC (p<0.001). The CO_2 emissions from both soil types responded similarly to temperature and water table treatments: they were significantly elevated by high temperature (p=0.01), especially in peat below the water table, and by lowering the water table (p=0.09). Cumulative CH_4 emissions (Fig. 5.2B) were increased by high temperature in PMC (p<0.01), but decreased by lowering the water table

(p<0.01) in both soil types. Similar to CO_2, cumulative N_2O emissions (Fig. 5.2C) were elevated by high temperature in both soil types (p=0.03).

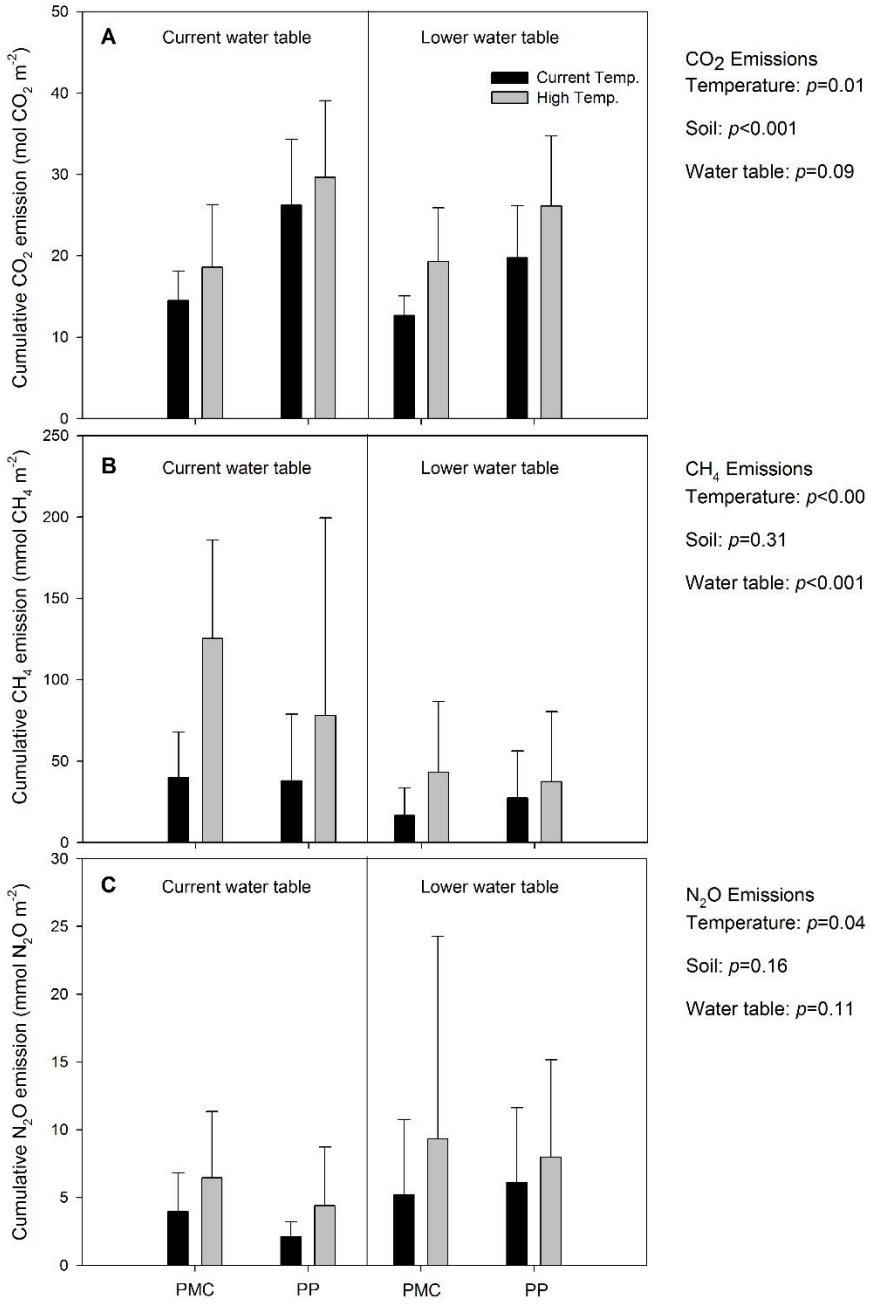

Fig. 5.2. Cumulative GHG fluxes over 28d incubation in two soil types under four temperature-water table treatments. A) CO_2 fluxes; B) CH_4 fluxes; C) N_2O fluxes. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

Temperature increased CO_2 and CH_4 concentration in all depths (p<0.02, except CO_2 concentration above the water table; Fig. 5.3 & 5.4). There was no interaction of temperature and soil types for CO_2 and CH_4 concentration. However, in peat submerged in water, PP had higher CO_2 concentration than PMC (p<0.01; Fig. 5.3). Moreover, Low water table treatment had lower CO_2 concentration in Surface (p=0.05) and greater CH_4 concentration in Surface and Above (right above water, p<0.01).

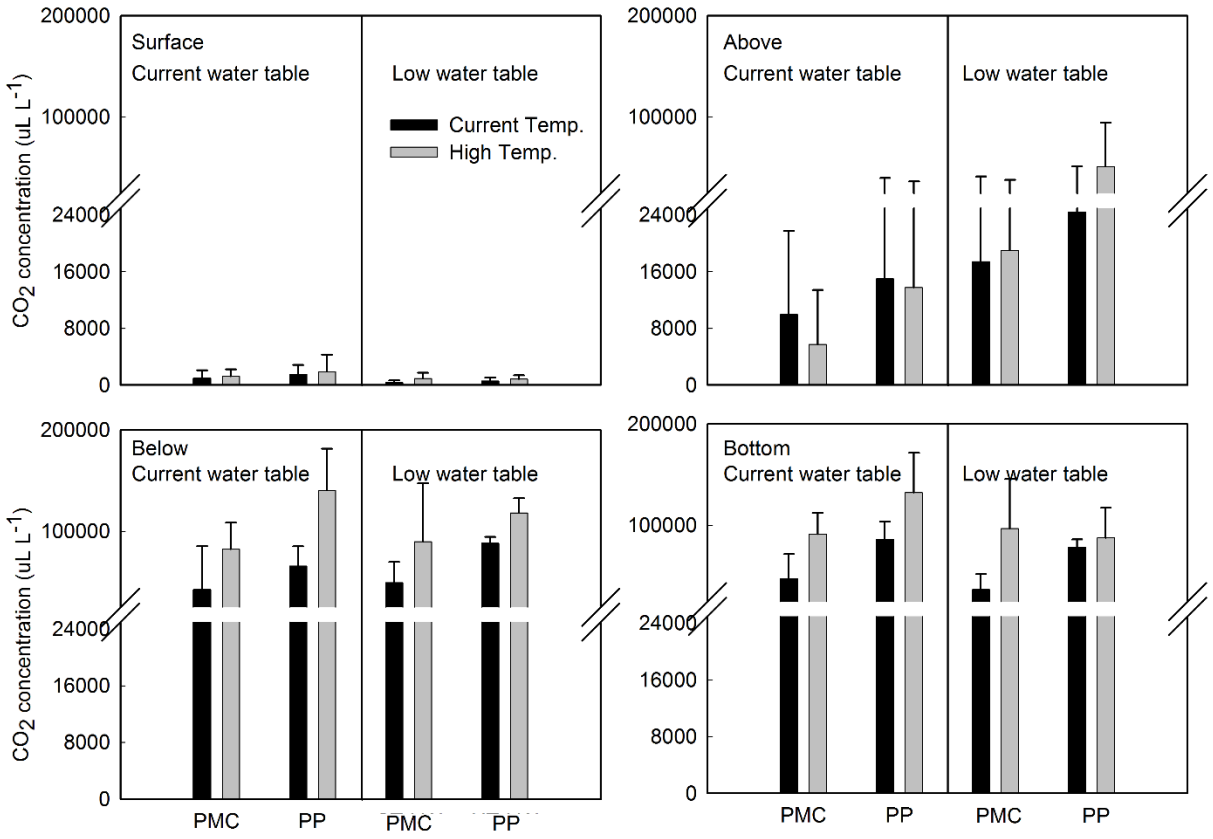

Fig. 5.3. Carbon dioxide concentration changes on day 28 in four different depths in two soil types under four temperature and water table treatments. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

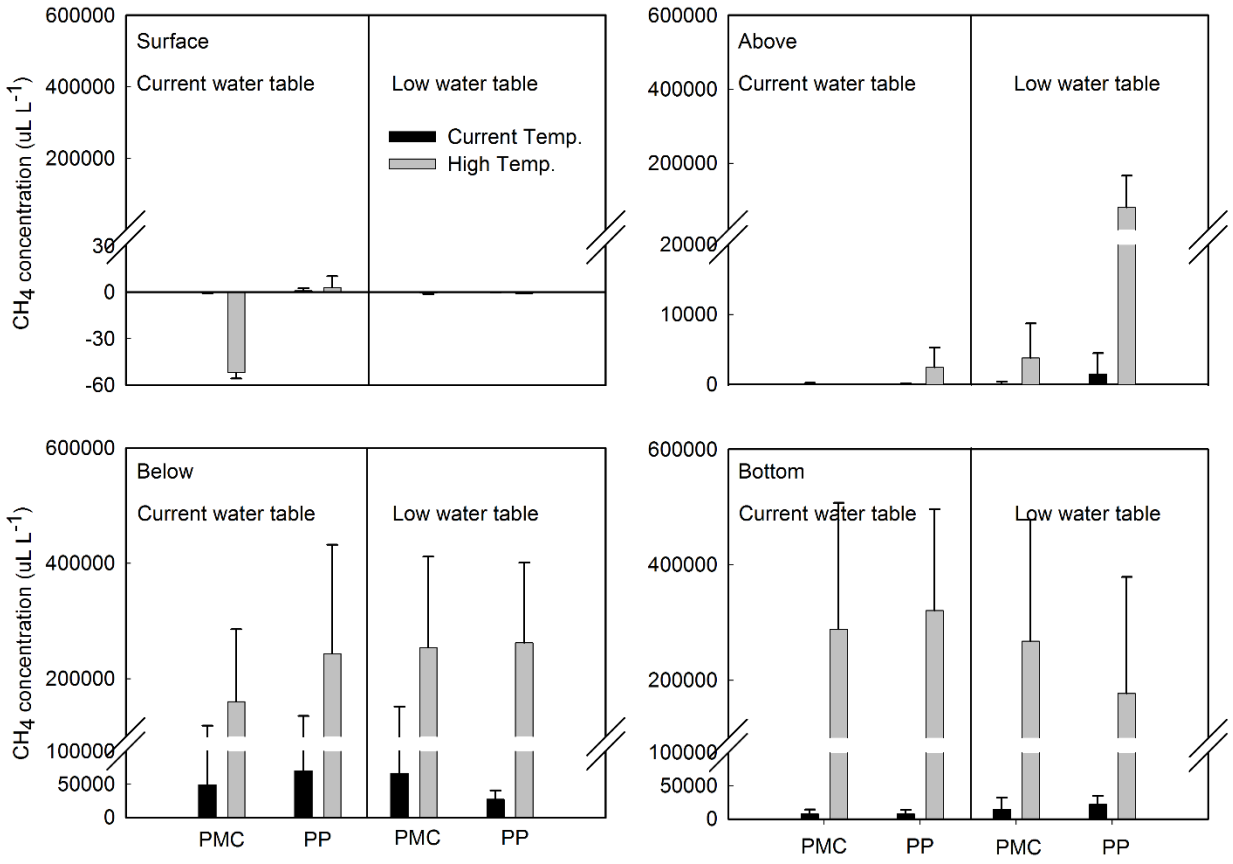

Fig. 5.4. Methane concentration changes on day 28 in four different depths in two soil types under four temperature and water table treatments. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

As was the case for CO_2 and CH_4, N_2O concentration was significantly elevated by high temperature at most depths (Above: p=0.03, Below: p=0.05, Bottom: p=0.02; Fig. 5.5); the exception was for Surface (p=0.95). Soil types also affected N_2O concentration at all depths except Surface (Above: p=0.11, Below: p=0.07, Bottom: p=0.01; Fig. 5.5). Unlike CO_2 and CH_4, N_2O concentration showed a clear interaction of soil type and temperature for the top three depths, especially right above and below water table, where temperature's positive effect on N_2O concentration was more significant in PP than in PMC (Above: p=0.14, Below: p<0.01; Fig. 5.5).

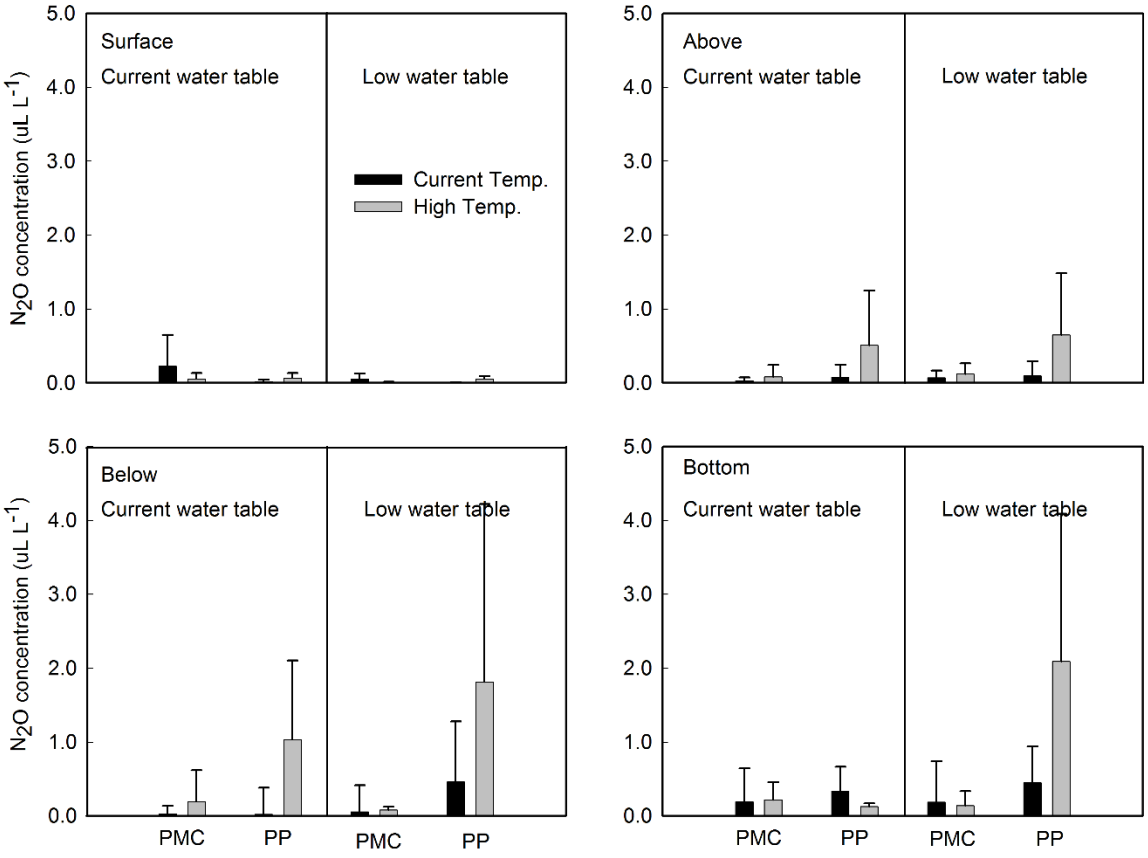

Fig. 5.5. Nitrous oxide concentration on 28d of incubation in four different depths of two soil types under temperature and water table treatments. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

5.5.2 Biogeochemical processes rates

After 28 d, potential CO_2 production decreased with depth (p<0.01), while CH_4 production increased with depth as expected (p<0.01; Fig. 5.6 & 5.7). There was no interaction of temperature and soil types for CO_2 and CH_4 production. The only differences were that CO_2 production from Background cores was higher than the treated cores at Surface and Above (p<0.01, Fig. 5.6) and that CH_4 production increased under high temperature treatment in Below (p<0.01) and showed increasing trend with temperature in Bottom (p<0.15, Fig. 5.7).

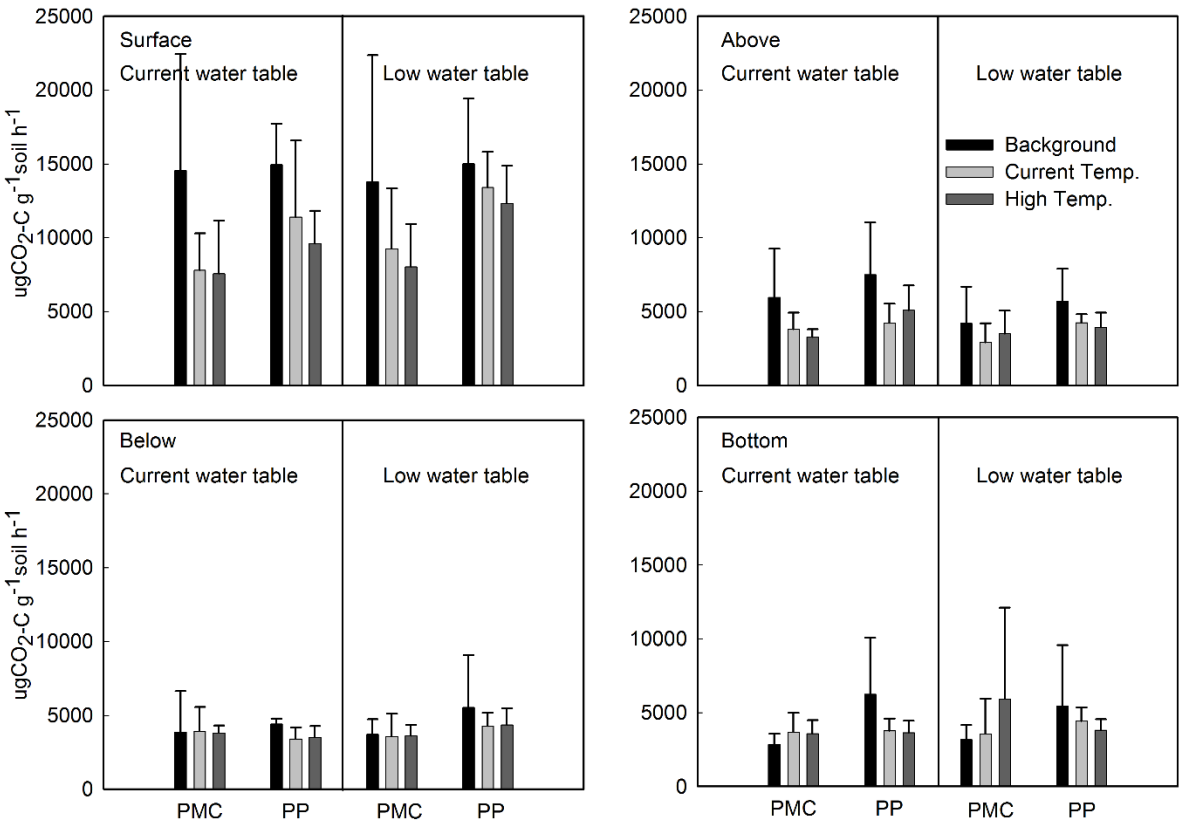

Fig. 5.6. Carbon mineralization rate (or net CO_2 production rate) over 24 h aerobic incubation in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

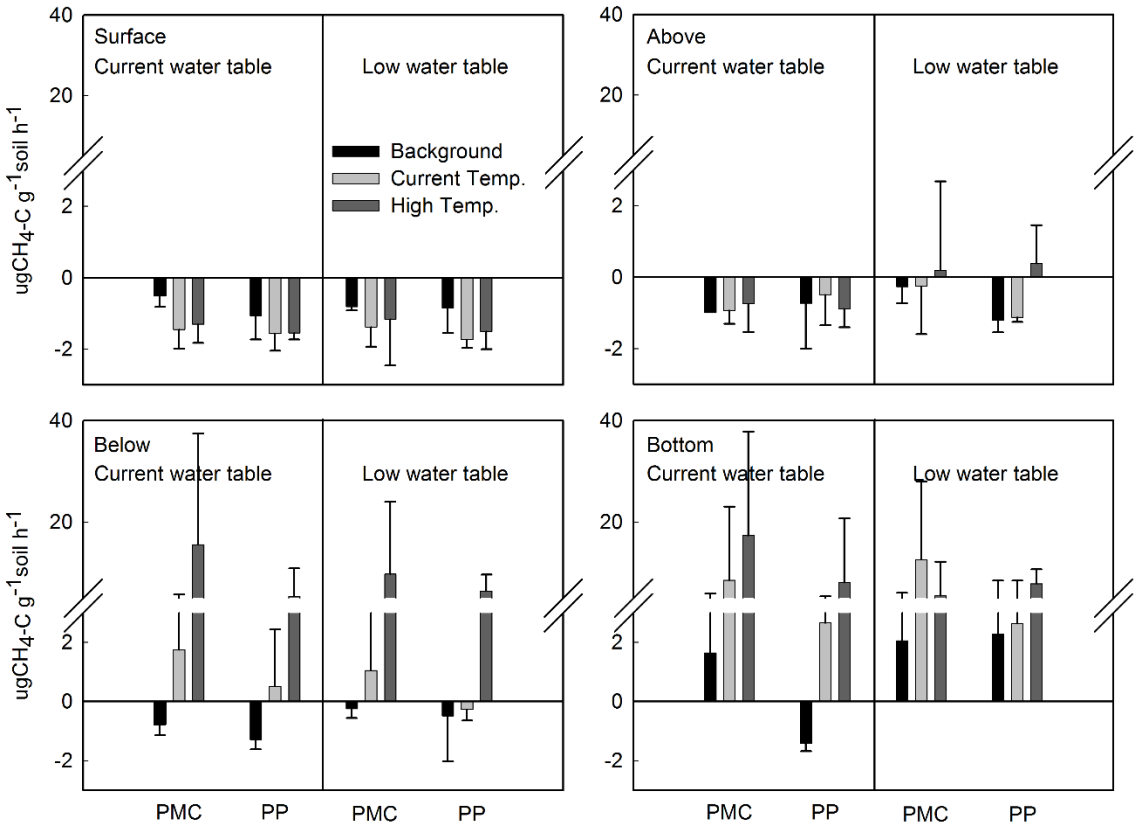

Fig. 5.7. Net CH_4 production rate over 24 h aerobic incubation in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

Potential N_2O production decreased with depth, with net production above the water table and net consumption below the water table. High temperature cores showed higher N_2O production rates than the Background core at Surface (p= 0.05) and Above (p<0.01), whereas Bottom high temperature cores had lower N_2O production rates than the Background core (p<0.01). The impact of soil type was detected at Above and Below, where N_2O production rates were higher in PP than in PMC (Above, p<0.01; Below, p=0.03). In Above, the positive effect of temperature on N_2O production was greater in PP than PMC, especially under current water table (Fig. 5.8). Similar to N_2O production rates, gross nitrification rates decreased with depth (p<0.01) (Fig. B5). Soil types also impacted gross nitrification rate; they were higher in PMC than PP at all depths except for Surface (p<0.05).

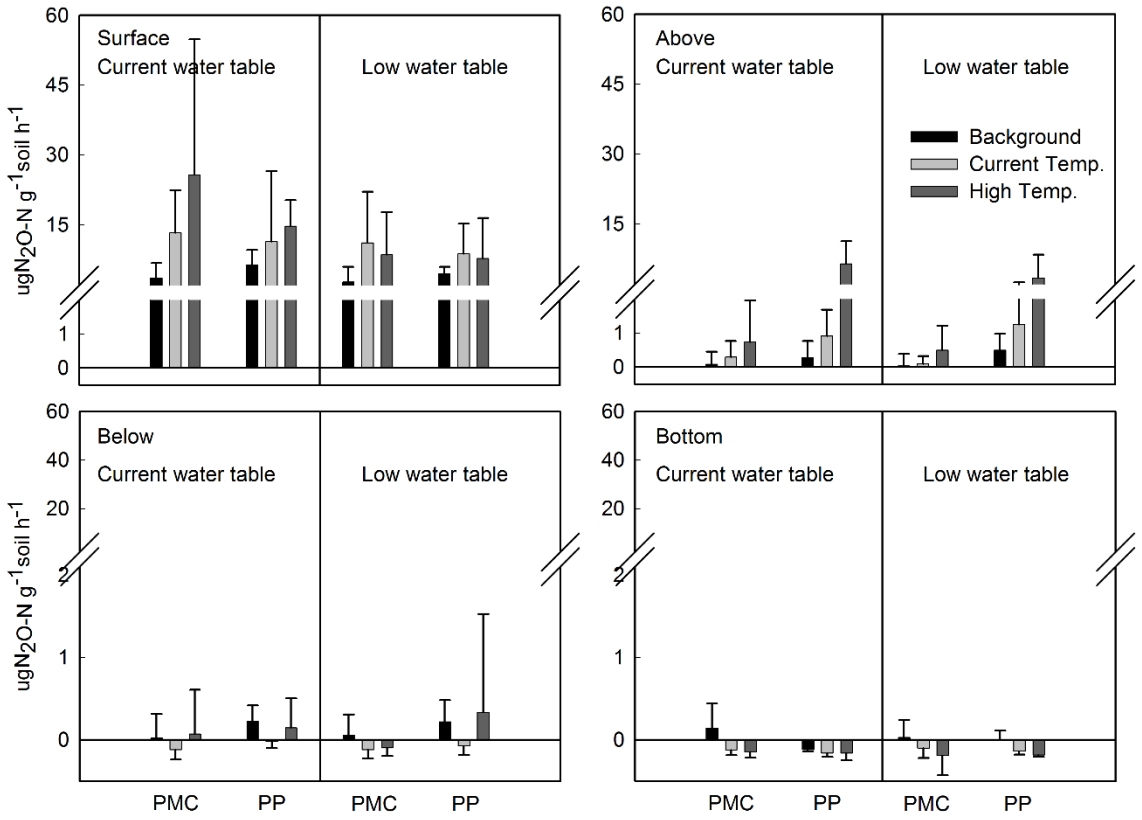

Fig. 5.8. Net N_2O production rate over 24 h aerobic incubation in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

5.5.3 Apparent enzyme activation energy

Apparent enzyme activation energy (E_a) of CO_2 and CH_4 production and consumption did not change significantly before versus after incubation (Fig. B.6). The only tendency were that below water-table E_a of CH_4 consumption increased after incubation in PP (p=0.15, Fig. B.6B) and E_a of CO_2 production decreased after high temperature incubation (p=0.14, Fig. B.6C).

Apparent enzyme activation energy of N_2O production was greater at high temperature (p<0.01) and at depth Above (p=0.10). In addition, an interaction effect of soil type and temperature treatment was indicated by a greater increase in N_2O generation E_a in PMC (Above: increased by 71.1 kJ mol^{-1}, Below: 42.8 kJ mol^{-1}) than PP (Above: 28.2 kJ mol^{-1}, Below: 17.4 kJ mol^{-1}) after the high temperature treatment (Fig. 5.9A). For N_2O consumption, the only

significant effect was found at Below, where high temperature treated cores had higher E_a (p=0.01) than Background cores in PMC (Fig. 5.9B).

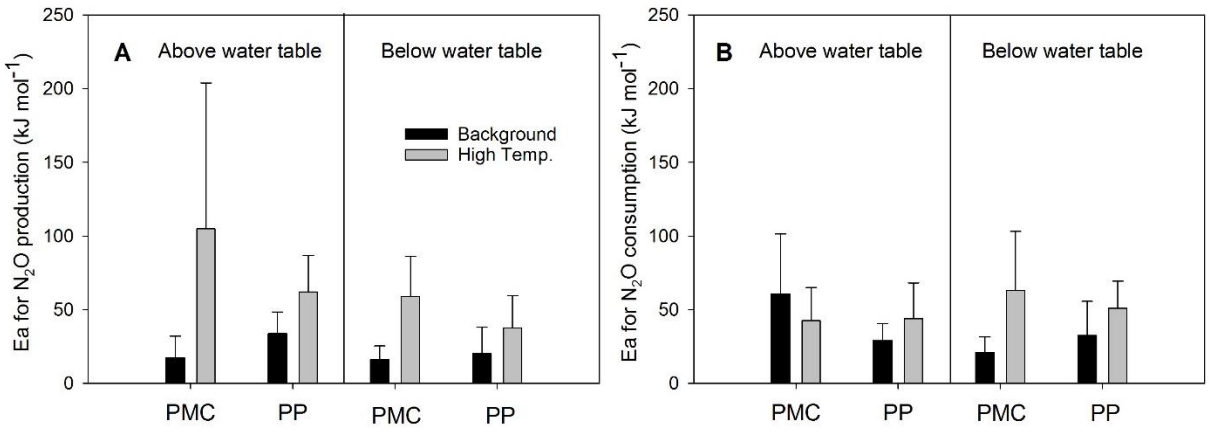

Fig. 5.9. Apparent enzyme activation energy (E_a) for N_2O production and consumption from peat 5 cm above and 5 cm below water table with and without 28d high temperature incubation in two soil types. A) E_a for N_2O production; B) E_a for N_2O consumption. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

5.5.4 Bacterial community structure

Different soil types showed differences in relative microbial community abundance (Fig. 5.10). For example, *Alphaproteobacteria* (p=0.018), *Gammaproteobacteria* (p=0.005), *Acidobacteria-Gp4* (p<0.001) and *Spartobacteria* (p<0.001) were more abundant in PP, whereas *Bacteroidetes*-unclassified (p<0.001), *Actinobacteria* (p=0.001) and *Deltaproteobacteria* (p=0.001) were more abundant in PMC. Compared with background cores, high temperature showed greater relative abundance of *Bacteroidetes*-unclassified (p<0.001) and *Verrucomicrobia-Subdivision3* (p<0.001). On the other hand, less relative abundance of *Alphaproteobacteria* (p=0.021), *Betaproteobacteria* (p=0.042) and *Gammaproteobacteria* (p=0.036) were detected under high temperature treatment. In addition, for *Gammaproteobacteria* and *Betaproteobacteria*, temperature treatment only affected their abundance in PMC.

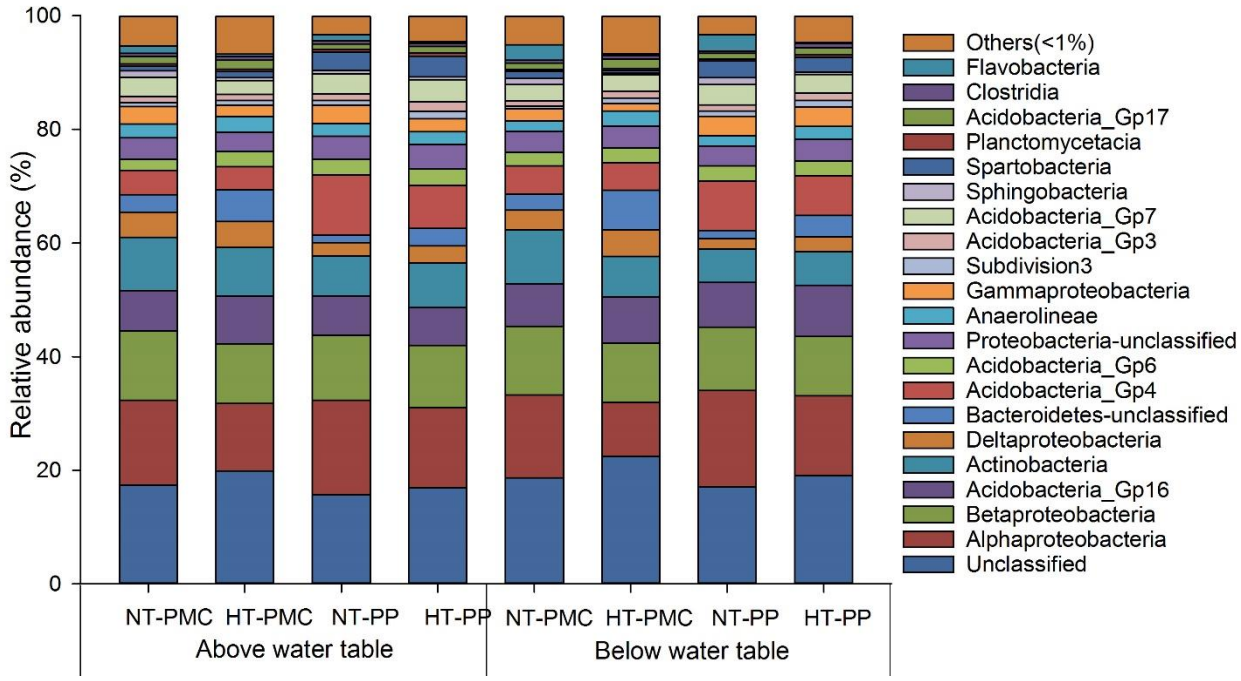

Fig. 5.10. Relative abundance of the dominant classes in peat from 5 cm above and 5 cm below water table with (HT) and without (NT) 28d high temperature incubation in two soil types. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

Nonmetric multidimensional scaling (NMDS) analysis of bacteria communities revealed a strong clustering according to soil type (Fig. 5.11). The permutation-based MANOVA also suggested an impact of high temperature treatment on bacterial community structure ($F=3.136$, $p=0.002$). Temperature not only affected bacteria communities, but also increased alpha diversity in all samples (Fig. B.9). However, microbial community structures were not significantly different between depths ($F=1.681$, $p=0.077$, Fig. 5.11).

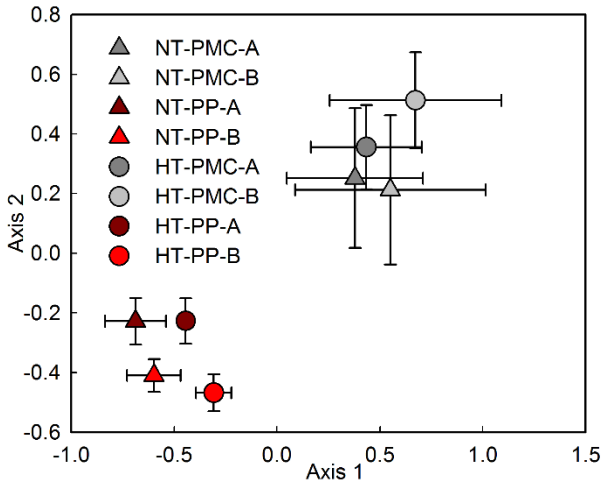

Fig. 5.11. Nonmetric multidimensional scaling (NMDS) analysis (final stress = 0.123, MRPP for Soil type, A=0.27, *p*=0.00, MRPP for temperature treatment, A=0.03, *p*=0.017,) of microbial communities of peat samples from 5 cm above (A) and 5 cm below water table (B), with (HT) and without (NT) 28d high temperature incubation in two soil types. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

5.6 Discussion

This study supports the notion that changes in CO_2, CH_4 and N_2O production are the most important factors influencing peatlands feedback to climate warming conditions (Blodau, 2002). This research also confirmed that increasing temperature resulted in increasing GHG cumulative emissions and concentrations at depth in peat soils. More importantly, it was found that mineral sediments affect GHG emission and concentrations. For example, the increasing N_2O concentrations under higher temperature showed an interaction with the presence of mineral sediments, as discussed below. Mineral sediments also affected CO_2 emission and concentrations at depth, but the effect of mineral sediments on CO_2 did not have an interaction with the temperature effect.

5.6.1 Impact of temperature and water table on CH_4

Methane emissions in northern peatlands account for 4-10% global CH_4 emissions (Mikaloff Fletcher et al., 2004). In this study, high temperature increased cumulative emission of CH_4 from headspace and CH_4 concentration from submerged samples (Figs. 5.2, 5.4). This is in line with previous findings (Dinsmore et al., 2009; Smith et al., 2003; Treat et al., 2014; Updegraff et al., 2001), as higher temperature encourages microbial activity (Davidson and

Janssens, 2006). However, the lack of significant differences in E_a implies that the methanogen community structure was less likely to change as a result of warming. When compared with other laboratory studies, CH_4 emission rates in this study (Table 5.4) were within the reported range. For CH_4 concentrations (Table 5.5), our current temperature (15°C) results were relatively high, but still within the reported range. However, under the high temperature treatment (25°C), CH_4 concentrations from below the water table (Table 5.5) were greater than all the referenced data. This is reasonable, as none of the listed studies were incubated at temperature as high as 25°C. The studies focusing on CH_4 concentration at depth (Table 5.5 or others), were usually looking at changes in water table rather than increasing temperature; therefore, the temperature used in these studies were not elevated.

Water table also affected CH_4 fluxes: as expected, lowering the water table decreased CH_4 fluxes as CH_4 production requires strictly anaerobic conditions (Deppe et al., 2010; Holmes et al., 2014; Jungkunst et al., 2008). Interestingly, when comparing the cumulative CH_4 emission from current temperature – current water table treatment (39.06 mmol CH_4 m^{-2}) with the high temperature – low water table treatment (40.27 mmol CH_4 m^{-2}), there were no differences. In this study, the high temperature treatment was set to 25°C to observe the maximum potential change. This resulted in a more intensive temperature increase (2-3°C higher than maximum daily temperature in summer) than is expected (2-3°C higher than average temperature) in central North America (IPCC, 2007). Therefore, CH_4 emissions under actual climate change scenario are likely to be even lower than we measured, which indicated CH_4 emissions might be decreased by warmer and drier conditions. This prediction is consistent with the finding in northern high altitude peatlands in which warmer and drier conditions could mitigate CH_4 emissions (Wang et al., 2010; Yang et al., 2014).

Table 5.4. Comparison of CH_4 emission rates from peat in laboratory and field studies.

Study	Type	Peat type	Water table (cm)	Air temperature (°C)	CH_4 emission (mg m^{-2} d^{-1})
This study	Laboratory	fen	-25/-40	15	546
This study	Laboratory	fen	-25/-40	25	1268
Aerts and Ludwig (1997)	Laboratory	fen	0~-10	20	240-1920
Dinsmore et al. (2009)	Laboratory	fen	-5~-35	5-10	0.46-4.58
Moore and Dalva (1993)	Laboratory	bog	0	22.6	3110
Yang et al. (2013)	Laboratory	freshwater marsh	-8.5	16.6	178.32
Yang et al. (2013)	Laboratory	freshwater marsh	-18.4	17	131.04
Yang et al. (2013)	Laboratory	freshwater marsh	+9.4	15.8	645.6
Danevčič et al. (2010)	Field study	drained fen	-53.2	10	0.96
Jungkunst and Fiedler (2007)	Field study	undrained bog	0~-6.3	8.5	37.0~139.9

Table 5.5. Comparison of CH_4 concentration from peat in laboratory studies.

Study	Type	Peat type	Water table (cm)	Air temperature (°C)	Depth (cm)	CH_4 concentration (ppmv) †
This study	Laboratory	fen	-25/-40	15	5 cm below surface (-5cm)	1
This study	Laboratory	fen	-25/-40	15	5 cm above water table (-20/-35cm)	769
This study	Laboratory	fen	-25/-40	15	5 cm below water table (-30/-45cm)	19722
This study	Laboratory	fen	-25/-40	15	5 cm above bottom (-55cm)	14721
This study	Laboratory	fen	-25/-40	25	5 cm below surface (-5cm)	-0.1
This study	Laboratory	fen	-25/-40	25	5 cm above water table (-20/-35cm)	6504
This study	Laboratory	fen	-25/-40	25	5 cm below water table (-30/-45cm)	75968
This study	Laboratory	fen	-25/-40	25	5 cm above bottom (-55cm)	81403
Beckmann and Lloyd (2001)	Laboratory	fen	0	19	-18	32465
Jungkunst et al. (2008)	Laboratory	fen	-5	15-25	-10	3131
Jungkunst et al. (2008)	Laboratory	fen	-5	15-25	-20	3468
Jungkunst et al. (2008)	Laboratory	fen	-5	15-25	-30	2487
Jungkunst et al. (2008)	Laboratory	fen	-20	15-25	-10	4
Jungkunst et al. (2008)	Laboratory	fen	-20	15-25	-20	1212
Jungkunst et al. (2008)	Laboratory	fen	-20	15-25	-30	1821
Jungkunst et al. (2008)	Laboratory	fen	-40	15-25	-10	2
Jungkunst et al. (2008)	Laboratory	fen	-40	15-25	-20	2
Jungkunst et al. (2008)	Laboratory	fen	-40	15-25	-30	108
Deppe et al. (2010)	Laboratory	alpine wetland	-20~-30	20	-11	17
Deppe et al. (2010)	Laboratory	alpine wetland	-20~-30	20	-30	3588 ‡
Deppe et al. (2010)	Laboratory	bog	app. -20	20	-11	3
Deppe et al. (2010)	Laboratory	bog	app. -20	20	-25	12849 ‡

† Data from the listed references were converted to equivalent units according to Fiedler et al. (2005)

‡ Maximum measured concentration; others are average concentration during study periods.

5.6.2 Impact of temperature and soil types on CO_2

Although CH_4 emissions might decrease in response to climate warming, northern peatlands may still be a C source as CO_2 emission might increase in a warmer and drier climate (Belyea and Malmer, 2004). My results indeed showed that high temperature increased both cumulative CO_2 emission and CO_2 concentrations from below the water table (Fig. 5.3). However, above the water table, the absence of increased CO_2 concentrations or a change in E_a does not necessarily indicate that CO_2 production was not stimulated by high temperature at this depth. It is more likely because gas diffusion from the soil column to atmosphere through water-saturated peat is slower than in unsaturated peat (Fang and Moncrieff, 1999); in addition, more roots in upper peat help to facilitate diffusion from the peat profile to the atmosphere (Joabsson and Christensen, 2001). Therefore, CO_2 from above the water table contributed to the higher cumulative CO_2 emission at the surface, whereas a greater proportion of CO_2 from below the water table accumulated in the profile (higher CO_2 concentrations at depth, Fig. 5.3). With less loss, the temperature effect on CO_2 concentrations was more clearly reflected below the water table. Moreover, although it was not as great as surface peat, potential CO_2 production from the subsurface was not negligible (Fig. 5.6). This is consistent with studies in both neotropical and northern high altitude peatlands, which suggested subsurface peat could contribute to a high proportion of CO_2 emission when exposed to warm, aerobic conditions (Liu et al., 2016; Wright et al., 2011). In addition, the high temperature treatment caused shifting of bacterial communities from copiotrophs to oligotrophs, indicated by an increase in relative abundance of oligotrophs and decrease of copiotrophs (Fig. 5.10). In particular, the most abundant copiotrophs, *Alphaproteobacteria*, decreased only below the water table. These results indicate that subsurface peat needs to be considered when studying C balances.

Soil type also affected CO_2 concentration in the bottom two depths: PP>PMC. Peat materials in the bottom two depths in PMC were more affected by mineral sediments. Calcareous sediments in PMC raised the pH to approximately neutral (Wang et al., 2016b), which is desirable for decomposers (Ye et al., 2012). In addition, mineral sediments provided more ions and electron acceptors, which can promote decomposition (Broder et al., 2012). Therefore, peat materials in PMC, especially in peat closest to the mineral sediments, were more decomposed and had lower WEOC and TOC content than PP (Table 5.1). With more labile C, decomposition in PP was less restricted by peat quality (Reiche et al., 2010), which resulted in higher CO_2

emissions. On the contrary, there was little influence of mineral horizons on CO_2 emission for the top two depths. This is because upper peat, especially surface peat, did not directly connect with mineral sediment and the C source for both soil types is mainly from aboveground vegetation. With similar dominant vegetation (sedges), CO_2 emissions from upper peat were similar for both PP and PMC.

5.6.3 Interaction impact of temperature and soil type on N_2O

Nitrous oxide emissions (current temperature: 96 μg m^{-2} h^{-1}, high temperature: 210 μg m^{-2} h^{-1}) in this study are within a similar range to other peatland studies (20 - 653 μg m^{-2} h^{-1}) (Danevčič et al., 2010; Jungkunst et al., 2008). Nitrous oxide concentrations are generally low compared to CH_4 and CO_2 (Marushchak et al., 2011). However, high N_2O production potential is expected to occur in response to global warming because high temperatures increase microbial abundance and activity and enhances soil organic matter mineralization to provide more inorganic N (Elberling et al., 2010). As expected, high temperature increased cumulative N_2O emission and concentration, but the response of N_2O concentration was different in PMC and PP: immediately above and below the water table, high temperature increased N_2O concentration significantly more in PP than in PMC (Fig. 5.5). In addition, a similar pattern was found in N_2O production from Above, where N_2O production in PP was more stimulated by the high temperature treatment than in PMC (Fig. 5.8). There are several possible explanations for this interaction.

The first and most important possibility is that there was more labile C (i.e., higher WEOC, $p<0.05$, and less decomposed mosses, Table 5.1) in PP than PMC. Labile C is known to stimulate microbial activity. In particular, when temperatures increase, lower apparent enzyme activation energy is required, and labile C is more readily used (Davidson and Janssens, 2006). It has been found that with enough inorganic N, adding labile C stimulated N_2O production (Liang et al., 2015; Wang et al., 2014), as denitrification is induced by C mineralization. In this study, the inorganic N substrate pool was sufficient and was even greater (Figs. B.10, B.11; especially NH_4^+-N, $p<0.01$) in PP. Therefore, N_2O production in PP responded more significantly to increasing temperature.

Secondly, higher E_a indicates more energy is required to process a reaction (Davidson and Janssens, 2006). Materials with poor substrate quality are excepted to have higher E_a and be

more sensitive to temperature changes (Davidson and Janssens, 2006). After the high temperature treatment, E_a of N_2O generation from PMC showed a greater increase than from PP (Fig. 5.9A). This suggested that PMC needs more energy to produce N_2O after high temperature treatment, as peat materials in PMC are less degradable than PP. Therefore, when temperature increases, PMC is less likely to produce as much N_2O as PP, as shown in Fig. 5.8 - Above.

Thirdly, in addition to labile C content, different E_a is also an indication of different microbial community structures (Sinsabaugh, 1994). Therefore, another possibility for the soil type responses of N_2O to high temperature was that microbial community structures were different in the two soil types and therefore responded differently after high temperature treatment. Differences in microbial community structures in these two soil types (Fig. 5.11) are mainly due to the different relative abundance of functional groups: copiotrophs and oligotrophs (Fig. 5.10). As PP had more labile C than PMC, the relative abundance of copiotrophs (*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Acidobacteria-Gp4*, etc.) was greater in PP, whereas the relative abundance of oligotrophs was greater in PMC (*Bacteroidetes*-unclassified, *Actinobacteira* and *Deltaproteobacteria*, etc) (Bastian et al., 2009). As discussed above, bacteria communities also shifted from copiotrophs to oligotrophs after the high temperature treatment. Notably, *Betaproteobacteria* and *Gammaproteobacteria* significantly decreased after high temperature treatment in PMC, but not in PP. This reflects the lower initial labile C content in PMC, which was more easily depleted at the higher temperature. The switch of copiotrophs to oligotrophs could also result in lower C use efficiency (Fierer et al., 2007); correspondingly, microbial activity is likely to be limited and results in lower N_2O production.

Lastly, lower pH is known to inhibit dinitrogen oxide reductase, which is responsible for reduction of N_2O to N_2 (Weslien et al., 2009); therefore, this inhibition contributes to N_2O accumulation. It has been found that although denitrification rate decreases with lowering pH, the N_2O to N_2 ratio increases and N_2O production rate decreases significantly by 0.5 pH unit over range of 4-6.5 (Van den Heuvel et al., 2011). In our study, pH was lower in PP (5.62) than PMC (6.05). Therefore, when incubated at high temperature, microbial activity was stimulated in both PMC and PP, but the lower pH in PP likely inhibited N_2O reduction and enhanced N_2O accumulation. The N_2O production below the water table did not show an interaction effect of soil type and temperature. It is possible that peat submerged in water was very low in O_2

availability, and therefore, there would still be considerable amount of N_2O reduced to N_2 in PP despite the low pH.

Of all possible explanations for the greater increase of N_2O in PP than PMC, the greater labile C concentrations are key. Besides directly stimulating C and N cycling, labile C content also directly or indirectly affected E_a and microbial community structures.

5.7 Conclusion

High temperature significantly increased emissions of all three GHGs and GHG concentrations within the peat profiles. Lowering the water table decreased only CH_4 emissions. Soil types also affected GHG emissions and /or concentrations: PP had higher CO_2 emissions and concentrations than PMC; immediately above and below the water table, PP also showed higher N_2O concentrations than PMC. Unlike GHG, the bacterial communities were more affected by soil type than temperature. High temperature incubation increased the E_a only of N_2O production and resulted in a shift of microbial community structure, from copiotrophs to oligotrophs. There was no interaction effect of soil profile and temperature for GHG emissions from the headspace. However, N_2O concentrations right above and below the water table in PP were more affected by higher temperature. A similar effect was found in N_2O production just above the water table. This is likely due to increased E_a for N_2O generation and changes of microbial community structure from copiotrophs to oligotrophs under higher temperature, especially in PMC. In addition, the higher content of labile C in PP appears to have facilitated N_2O production; at the same time, lower pH in PP could decrease N_2O reduction to N_2 and accumulate N_2O. In contrast, the responses of CO_2 and CH_4 to high temperature were similar in both PMC and PP.

Overall, peat profiles with mineral horizons tended to produce less CO_2 and N_2O compared to peat without mineral horizons. In addition, the interaction of peat and mineral horizons minimized the increase of N_2O under increasing temperature. These findings suggest that mineral horizon should be carefully considered when predicting GHG responses under global warming. A better understanding of peatlands developed with underlying mineral sediments could be important in understanding feedback of GHG to global warming.

6. SYNTHESIS AND CONCLUSIONS

Northern peatlands play an important role in the global C and N cycles. In Canada, over 13000 km^2 are mountain peatlands (Cooper et al., 2012; Zoltai and Vitt, 1995). Mountain areas are known for geomorphic instability. Therefore, peat profiles developed in mountain areas are usually developed with shallow underlying and interrupted mineral sediments. Previous studies showed that mineral horizons in peat profile affect groundwater movement and chemistry (Chadde et al., 1998; Steinmann and Shotyk, 1997a), which are controlling factors on peat formation in peatland ecosystems. This ultimately led to the main objectives of this study: to investigate the effect of mineral horizons on 1) spatial soil properties distributions, 2) biogeochemical processes and 3) the response of GHG emissions and concentrations at depth to warming temperature. This study surveyed the mineral sediment distribution in Sibbald research wetland. Three organic soil types were identified: sedge peat/silty sediments/calcareous sediments (PMC), sedge peat/silty sediments/moss peat (PMP) and sedge peat/moss peat (PP). Key soil properties (θv, pH, TOC, and TN) were tested from different layers in 30 random sampling points from each of the three soil types. Then, the spatial distribution of soil properties in the peatland was characterized by universal kriging (Chapter 3). Based on spatial analysis, eight representative sampling points were selected for C and N cycling rates analysis (Chapter 3&4). Nitrification and C and N mineralization were determined by incubation under surface-like conditions. Before incubation, microbial community structure and C chemistry were measured by PLFA and FTIR spectroscopy. According to the findings from spatial soil properties and C and N cycling, PMC and PP were selected for the microcosm study which manipulated temperature and water table levels. GHG emissions and concentrations at different depth were measured during the microcosm experiment. Biogeochemical processes rates, E_a and microbial community structures were compared before and after microcosm experiment. The main findings can be summarized as follows.

6.1 Summary of findings

Spatial distributions of key soil properties (TOC, TN, pH and θv) in this mountain peatland were affected by mineral sediments via influencing water infiltration and ions diffusion and further influencing organic matter decomposition. However, the influence of mineral material was only notable at depth (Chapter 3).

The distribution of peat properties are related to biogeochemical processes (Chapin et al., 2003). In this study, mineral sediments also affected C and N cycling rates. The impact of mineral horizons on N cycling was mainly observed in peat adjacent to calcareous sediments, which provided HCO_3^-, increased pH to neutral level, promoted nitrification, and reduced lag phase in deeper peat (Chapter 3). For C cycling, stratified mineral horizons indirectly affected C mineralization rates via influencing both substrate quality and microbial community structures (Davidson and Janssens, 2006). Similarly, the effect of mineral sediments was mainly reflected in subsurface peat, but did not extend to surface peat (Chapter 4). Middle peat layers, directly adjacent to mineral sediments, were more decomposed (i.e., higher ratio of recalcitrant C to labile C) and showed higher stability, which could also cause highest microbial physiological stress in middle peat. Together these restricted the C mineralization rate in middle peat and resulted in similar C mineralization rates in middle peat and deep peat, although microbial abundance decreased significantly from middle peat to deep peat. In addition, stratified mineral horizon slowed infiltration of groundwater and organic acid from middle peat to deep peat, which influenced microbial community structure such as B:F and microbial community profiles. This further resulted in similar C mineralization rates in deep peat of PP and PMP, although PP had more WEOC.

In order to examine the impact of mineral sediments on GHG production under climate warming condition, a microcosm experiment was conducted: intact peat cores were collected from PMC and PP and subjected to temperature-water table treatments (Chapter 5). Temperature increased emission and concentration of all three gases as expected. Soil type only significantly affected CO_2 emission and concentration and N_2O concentration in subsurface peat. In addition, N_2O concentrations near the water table in PP were more affected by increasing temperature. Similar trend was found in N_2O production rate at 5 cm above water table. This is probably because of increased E_a for N_2O generation and changes of microbial community structure from copiotrophs to oligotrophs, especially in PMC, by high temperature. In addition, the higher content of labile C in PP facilitated N_2O production; at the same time, lower pH in PP could decrease N_2O reduction and build up N_2O accumulation. Overall, mineral sediment played an important role in peat profile development and affected key soil properties and substrate quality. These factors further influenced C and N cycling and microbial community structure at present and might also affect peat profiles' future responses to climate warming conditions.

6.2 Stratified mineral horizons and subsurface peat: pedological properties and climate change

Studies focused on continuous peatland indicated that soil properties and decomposition degrees varied in subsurface peat in both low latitude (Wright et al., 2011) and high latitude (Dorrepaal et al., 2009; Hodgkins et al., 2014) peatlands, as paleo-climate changed. When there are mineral sediments present in the peat profile, the variation with depth could be even greater. It has been found that in peatlands with complex stratigraphy, mineral sediments can provide nutrients and electron acceptors, which can increase decomposition of nearby peat (Broder et al., 2012). Peatlands in Rocky Mountain areas are usually developed with underlying and/or interbedded mineral sediments (Morrison, 2014). These mineral sediments could derived from paleopond sediments (Johnston, 2012), volcanic ash deposition (Zoltai, 1989) and autogentic deposition like marl (Churchill, 1962), etc. This study has clearly shown that by slowing water infiltration, mineral sediments resulted in a drier deep peat (Chapter 3) and a more decomposed and stabilized middle peat (Chapter 4). The substrate quality affected microbial communities: more decomposed material resulted in the highest physiological stress middle peat (Chapter 4) and more abundance of oligotrophs in PMC and more abundance of copiotrophs in PP (Chapter 5). Some sediments, like marl, increased pH in peat nearby and affected both N cycling rates (Chapter 3) and N_2O production (Chapter 5). Together these affected C and N cycling (Chapter 3 and 4) especially in subsurface peat, because subsurface peat is closer to mineral sediments and more easily accesses dissolved nutrient and ions and/or physical and chemical protection. On the other hand, surface peat mainly received fresh organic C from living plants and is more vulnerable to landform influence. Therefore, instead of being affected by mineral sediment, surface peat in this study is affected by geomorphic factors and vegetation.

Subsurface peat has been overlooked in earlier studies due to the low temperature and water logged conditions. Nowadays, with the growing number of studies on microorganisms and increasing need to understand feedback of peatland to climate warming condition, researchers are paying more attention to subsurface peat, recognizing that there are still large amounts of microbial biomass in deep peat (Lin et al., 2014 and Chapter 4). Under climate warming conditions (increased temperature, and lowered water table), the subsurface peat that has been preserved for years could be vulnerable to decomposition. However, the long term response of old organic matter buried in depth to increasing temperature remains unclear.

Recent studies found that higher temperature accelerated CO_2 and CH_4 production from subsurface peat in subarctic peatland (Chowdhury et al., 2015; Dorrepaal et al., 2009; Liu et al., 2016). However, these results were from studies on continuous peat. Since mineral sediments are important regulators in soil properties and C and N cycling in peat at depth, it is highly likely that mineral sediments could affect GHG production and further the feedback of complex peat profiles to climate change. A previous study indicated that substrate quality is the main factor regulating GHG production (Wright et al., 2011). This is consistent with our findings that subsurface peat from PMC had lower CO_2 concentration and production rate than PP (Chapter 5), as subsurface peat in PMC experienced greater decomposition during peat formation and at present, is physio-chemically protected by mineral sediments (Chapter 4).

Peatlands are important sources of CH_4 emission because of their waterlogged condition. Northern peatlands alone account for up to 4~10% of the global CH_4 emissions (Mikaloff Fletcher et al., 2004). Methane emissions are regulated by both hydrological conditions and temperature (Dinsmore et al., 2009; Moore et al., 2011). In our study, cumulative CH_4 emission under current temperature – water table condition was not different from that in climate warming condition (higher temperature and lower water table treatment), even with an extreme temperature increase (10 °C). At the same time, in subsurface peat, even with different substrate quality and different microbial community structures, the response of CH_4 production to increased temperature was similar. This is consistent with the findings in Yvon-Durocher et al. (2014) which showed that temperature dependence of CH_4 production is consistent in different strains of methanogens, different communities and different ecosystems. Therefore, it is likely that when studying CH_4 emission, it is not essential to consider subsurface profile.

Although N is usually limited and N_2O production is low in peatlands (Limpens et al., 2006), high N_2O production potential is expected to occur under global warming scenario (Elberling et al., 2010). For peatlands with complex stratigraphy, like our research site, we found that mineral sediments are likely to mitigate N_2O production from subsurface, as mediated by C quality. Therefore, when evaluating N_2O changes in a global warming condition, it is important to study the effect of complex peat profile (Chapter 5). In Chapter 3 we found that mineral sediments, especially the calcareous mineral sediments, mitigate the lag phase of N mineralization and nitrification in subsurface peat, i.e., N cycling rates were higher in PMC than PP at the beginning (before 28 days). However, higher N cycling rates did not necessary result in

higher N_2O production. Moreover, the 28d incubation period is enough to eliminate the lag phase effect. In addition, although N_2O production was enhanced under high temperature, microbial community structure was not much changed compared to N_2O production. This is consistent with the previous finding that N cycling rates changed but not enzyme and microbial community structures (Weedon et al., 2012).

6.3 Future Research

This research provides a fundamental understanding of mineral sediment effects on soil properties and biogeochemical processes in northern mountain peatland. Moreover, it provides insight into how complex peat profiles react in simulated climate warming condition. Complex peat profiles have been studied from geological and hydrological perspectives. This study filled in the knowledge gap from pedological perspective. A better understanding of complex peat profiles and their role in a changing climate will be particularly important for evaluating soil C sequestration and nutrient dynamics under climate warming scenarios in northern peatlands.

For this work, it could be fruitful to explore whether the pathways of CH_4 and N_2O production are affected in these complex peat-mineral systems with stable isotope labeling techniques. From a microbial perspective, this work only analyzed the universal 16S rDNA genes for bacteria; it would be interesting to investigate the changes of functional genes for RNA, which represent the functional living microorganisms. In addition, in the microcosm study (Chapter 5), only underlying mineral sediments were studied. However, the interbedded mineral sediments might be also important and worth consideration, especially where the water table moves across the interbedded mineral sediments. Future microcosm studies might decrease water table and/or increase temperature gradually over incubation time to better simulate the actual environmental changes in field during summer. Another option would be to provide several short periods of extreme changes of temperature and water table, as climate change scenarios suggest not only increasing temperature but also more extreme weather events.

The findings in this work were based on only one peatland. More study is needed on biogeochemical processes in complex peatland soil profiles across high latitude to low latitude. A careful evaluation should be done about how widespread and important this influence would be. In particular, long term experiments under laboratory incubation and/or in field should be carried out to verify the response of GHG production in complex peat profile to climate change.

Moreover, this research did not consider vegetation and climate change interaction, which has been found to have important effects on C sequestration (Ward et al., 2015). Vegetation can affect GHG emission directly. For example, vascular plants with *aerenchyma* (hollow tissue) can not only directly help to transport GHG up to the atmosphere, but can also diffuse oxygen into rhizosphere (Colmer, 2003). The diffusion of oxygen is likely to promote CO_2 production, but inhibit CH_4 production. In addition, it has been reported that under global warming, vegetation growth and biomass might be increased, which can provide more fresh OC input into soil, cause a priming effect and stimulate GHG emission (Heimann and Reichstein, 2008).

Besides providing a better understanding on natural peatlands, the interaction of mineral sediments could have some implications on constructed peatlands in mining area reclamation, and is worthy of further study. In Alberta, some reclamation approaches involve establishing peat materials on top of mineral sediments to restore the oil sand mining region (Borkenhagen and Cooper, 2016). The mineral sediments in constructed peatlands might serve similar functions as the mineral sediments in this study, i.e., influence groundwater movement and groundwater chemistry and in turn, affect C and N cycling rates. In addition, when comparing pedological and biogeochemical differences of constructed peatlands and natural peatlands, peat stratigraphy should be considered to select the comparable peatlands.

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APPENDIX A. PROPERTIES OF MINERAL SEDIMENTS AND PEAT SAMPLES

Table A.1. Major-element chemistry of the sediments (weight%), measured by X-ray fluorescence (XRF).

	Silty sediments in PMC†	Silty sediments in PMP†	Calcareous sediments in PMC
Na_2O	3.78±0.20	3.68±0.34	0.24±0.15
MgO	0.69±0.00	0.71±0.05	1.17±0.14
Al_2O_3	15.73±0.57	15.8±0.73	3.81±2.02
SiO_2	66.9±0.96	65.10±3.28	16.30±11.30
P_2O_5	0.12±0.01	0.15±0.03	0.07±0.01
K_2O	2.26±0.08	2.20±0.20	0.40±0.25
CaO	2.26±0.13	2.44±0.28	41.60±6.79
TiO_2	0.50±0.00	0.49±0.02	0.11±0.08
MnO	0.05±0.00	0.05±0.00	0.04±0.01
Fe_2O_3	2.92±0.10	2.59±0.32	0.62±0.32

† PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments/peat.

Table A.2. Particle size analysis.

	Sand%	Silt%	Clay%
Silty sediment in PMC†	20.82±2.13	74.26±1.88	4.92±0.31
Silty sediment in PMP†	23.09±0.6	71.14±0.75	5.77±0.61

† PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat.

Table A.3. Major physical and chemical properties of soil samples in each horizon of three soil types.

Soil types†	Horizon‡	θv (cm^3 cm^{-3})	pH	TN (%)	TOC (%)	C/N
PMC	SP	0.73 ± 0.29	6.27 ± 0.38	2.73 ± 0.35	40.02 ± 5.68	14.79 ± 1.79
	MP	1.00 ± 0.40	5.97 ± 0.37	2.39 ± 0.67	31.05 ± 9.39	13.16 ± 0.88
	M	0.70 ± 0.30	6.79 ± 0.51	0.39 ± 0.37	5.37 ± 4.74	29.18 ± 23.75
	C	0.63 ± 0.16	7.4 ± 0.07	0.15 ± 0.08	2.54 ± 0.79	59.59 ± 49.62
PMP	SP	0.86 ± 0.20	5.41 ± 0.33	2.93 ± 0.29	40.9 ± 6.44	14.45 ± 2.14
	MP	1.12 ± 0.42	5.52 ± 0.42	2.65 ± 0.36	38.82 ± 6.15	14.54 ± 1.35
	M	1.02 ± 0.70	6.01 ± 0.28	0.17 ± 0.31	2.92 ± 4.08	19.61 ± 13.89
	DP	0.52 ± 0.17	5.91 ± 0.56	2.28 ± 0.39	37.33 ± 5.38	16.17 ± 1.6
PP	SP	0.62 ± 0.16	5.9 ± 0.55	2.82 ± 0.4	43.99 ± 1.62	15.2 ± 2.5
	MP	0.73 ± 0.15	5.58 ± 0.22	2.97 ± 0.28	44.16 ± 1.98	15.08 ± 1.48
	DP	0.79 ± 0.23	5.41 ± 0.15	2.75 ± 0.3	45.83 ± 2.72	18.15 ± 4.85

† PMC: peat/silty mineral sediments/calcareous sediments, PMP: peat/ silty mineral sediments /peat, PP: sedge peat/moss peat.

‡ SP: surface peat, MP: middle peat, DP: deep peat, M: silty mineral sediment, C: calcareous sediments.

APPENDIX B. GHG EMISSION OVER 28 DAYS AND RELATED DATA

B1. Supplementary results

B1.1 Cumulative GHG emission and GHG concentration at different depth in day 28

Cumulative CO_2 emission from all samples kept increasing at similar speed with incubation time over the 28 d (Fig. B1A). Cumulative CH_4 emissions also increased with incubation time. Most samples incubated at high temperature started to increase CH_4 emission earlier, and cumulative CH_4 emission from samples incubated at current water table increased more aggressively (Fig. B1B). Cumulative N_2O emission from all samples kept increasing until day 14 when it came to a plateau (Fig. B1C).

When looking into CO_2 concentration along the peat profile, CO_2 concentration from 5 cm below surface decreased with time (p=0.000), while CO_2 concentration from the other three depths increased with time (p<0.05) (Fig. B2). For CH_4 concentration in depth, it showed similar pattern as CO_2, except that from 5 cm below surface did not significantly changed with time (Fig. B3). For N_2O concentration in different depths, the only change with incubation time was found from 5 cm below surface where its concentration decreased with time (p=0.005) (Fig. B4).

B1.2 Bacterial community structure

The OTUs belonged to 25 phyla and 79 classes and there are over 18% OTUs were unclassified at phylum level. *Proteobacteria* (35.0%) and *Acidobacteria* (23.3%) were the most abundant phyla across all samples. At phyla level, most phyla shown in Fig. B10 are different in PP and PMC. *Proteobacteria* (p=0.08), *Acidobacteria* (p=0.00) and *Verrucomicrobia* (p=0.00) are more abundant in PP than PMC. However, the unclassified, *Actinobacteria* (p=0.00), *Bacteroidetes* (p=0.001) and *Firmicutes* (p=0.001) are more abundant in PMC. In addition, incubated at high temperature also affected certain phyla relative abundance. For example, the relative abundance of *Proteobacteria* were decreased after incubated at high temperature (p=0.00), while unclassified showed the opposite trend (p=0.00).

B2. Supplementary tables

Table B.1. Average ion concentration in East Inlet and Bateman Creek.

Ions and pH	Average ion concentration (mg L^{-1})
HCO_3^-	192.00
CO_3^{2-}	6.33
Cl^-	0.33
OH^-	0.00
PO_4^{3-}	0.00
NO_3^-	0.07
NH_4^+	0.45
Ca^{2+}	36.00
Mg^{2+}	13.00
K^+	0.33
Na^+	5.67
SO_4^{2-}	3.50
pH	8.29

Table B.2. Significance levels from ANOVA test: effects of high temperature treatment, soil type and depth (above or below water table) on GHG concentration.

		Df	CO_2		CH_4		N_2O	
			F-value	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**
Surface	Temperature treatment (T)	1	4.748	0.035	21.344	0.000	0.003	0.954
	Soil types (S)	1	0.166	0.686	134.512	0.000	0.076	0.784
	Water table (W)	1	3.938	0.054	0.897	0.349	1.321	0.257
	T × S	1	0.206	0.652	106.357	0.000	7.416	0.010
	T × W	1	0.245	0.623	3.210	0.081	0.515	0.477
	S × W	1	0.662	0.421	464.514	0.000	0.791	0.379
	T × S × W	1	0.064	0.802	338.305	0.000	0.558	0.460
Above	Temperature treatment (T)	1	0.928	0.341	6.952	0.012	5.386	0.026
	Soil types (S)	1	0.044	0.835	45.657	0.000	2.557	0.118
	Depth (D)	1	0.041	0.840	0.025	0.876	1.342	0.254
	T × S	1	0.142	0.708	2.841	0.100	2.267	0.140
	T × D	1	3.928	0.054	0.640	0.429	1.205	0.279
	S × D	1	2.071	0.158	0.461	0.501	0.573	0.453
	T × S × D	1	0.104	0.749	33.026	0.000	0.389	0.537
Below	Temperature treatment (T)	1	24.142	0.000	55.168	0.000	4.177	0.048
	Soil types (S)	1	0.153	0.698	6.429	0.015	3.417	0.072
	Depth (D)	1	15.424	0.000	1.618	0.211	0.577	0.452
	T × S	1	1.374	0.248	2.539	0.119	10.097	0.003
	T × D	1	0.418	0.522	0.004	0.948	0.269	0.607
	S × D	1	0.137	0.713	1.994	0.166	3.389	0.073
	T × S × D	1	1.428	0.239	0.076	0.784	0.013	0.909
Bottom	Temperature treatment (T)	1	23.669	0.000	32.123	0.000	5.997	0.019
	Soil types (S)	1	3.207	0.081	0.342	0.562	8.793	0.005
	Depth (D)	1	11.840	0.001	0.421	0.520	3.876	0.056
	T × S	1	0.416	0.522	1.249	0.270	3.475	0.070
	T × D	1	2.229	0.143	0.661	0.421	4.346	0.044
	S × D	1	2.099	0.155	0.252	0.619	4.668	0.037
	T × S × D	1	2.649	0.111	0.544	0.465	5.563	0.023

Table B.3. Significance levels from ANOVA test: effects of high temperature treatment, soil type and depth (above or below water table) on GHG production rates.

		Df	CO_2		CH_4		N_2O	
			F-value	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**
Surface	Temperature treatment (T)	2	5.691	0.006	4.494	0.016	3.156	0.052
	Soil types (S)	1	8.628	0.005	0.015	0.905	0.000	0.989
	Water table (W)	1	1.687	0.200	2.648	0.111	2.283	0.137
	T × S	2	0.682	0.511	0.082	0.921	0.094	0.911
	T × W	2	0.382	0.685	0.017	0.983	0.345	0.710
	S × W	1	0.223	0.639	0.026	0.872	0.026	0.873
	T × S × W	2	0.109	0.897	0.333	0.718	0.027	0.974
Above	Temperature treatment (T)	2	7.169	0.002	1.070	0.351	7.554	0.001
	Soil types (S)	1	2.907	0.095	6.779	0.012	45.757	0.000
	Depth (D)	1	7.012	0.011	0.316	0.577	0.447	0.507
	T × S	2	0.805	0.453	1.428	0.250	11.420	0.000
	T × D	2	0.164	0.849	0.296	0.746	0.560	0.575
	S × D	1	0.068	0.796	1.894	0.175	2.005	0.163
	T × S × D	2	0.759	0.474	0.408	0.667	1.165	0.320
Below	Temperature treatment (T)	2	0.859	0.430	55.168	10.069	1.160	0.322
	Soil types (S)	1	0.907	0.346	6.429	0.173	4.797	0.033
	Depth (D)	1	1.106	0.298	1.618	2.889	0.151	0.700
	T × S	2	0.028	0.972	2.539	0.146	0.415	0.663
	T × D	2	0.708	0.498	0.004	1.147	0.103	0.902
	S × D	1	2.640	0.111	1.994	0.378	0.050	0.824
	T × S × D	2	0.010	0.990	0.076	0.462	0.049	0.952
Bottom	Temperature treatment (T)	2	0.729	0.488	1.366	0.265	8.896	0.001
	Soil types (S)	1	1.698	0.199	0.352	0.556	0.010	0.919
	Depth (D)	1	0.549	0.462	0.968	0.330	1.916	0.173
	T × S	2	3.153	0.052	1.546	0.224	0.306	0.738
	T × D	2	0.409	0.667	1.172	0.318	1.258	0.293
	S × D	1	0.166	0.686	0.925	0.341	0.680	0.414
	T × S × D	2	0.761	0.473	0.669	0.517	0.952	0.393

Table B.4. Significance levels from ANOVA test: effects of high temperature treatment, soil type and depth (above or below water table) on apparent enzyme activation energy (E_a) for CH_4, N_2O production and consumption and for CO_2 production.

		Df	CH_4		N_2O		CO_2	
			F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Production	Temperature treatment (T)	1	0.104	0.750	17.616	0.000	1.020	0.320
	Soil types (S)	1	0.177	0.678	1.130	0.296	0.609	0.441
	Depth (D)	1	1.783	0.194	2.945	0.096	2.705	0.110
	T × S	1	0.024	0.879	3.641	0.065	4.186	0.049
	T × D	1	0.264	0.612	1.387	0.247	0.179	0.675
	S × D	1	0.292	0.594	0.048	0.828	0.547	0.465
	T × S × D	1	0.471	0.499	0.493	0.487	0.113	0.739
Consumption	Temperature treatment (T)	1	0.000	0.997	6.904	0.014	NA	NA
	Soil types (S)	1	3.370	0.078	0.008	0.928	NA	NA
	Depth (D)	1	2.517	0.124	0.143	0.709	NA	NA
	T × S	1	2.971	0.096	0.043	0.838	NA	NA
	T × D	1	7.672	0.010	4.115	0.053	NA	NA
	S × D	1	0.618	0.439	0.001	0.972	NA	NA
	T × S × D	1	0.284	0.599	2.857	0.103	NA	NA

Table B.5. ANOVA analysis of the diversity indexes.

	Df	Chao		Shannon		Simpson	
		F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Temperature treatment (T)	1	30.656	0.000	13.381	0.001	8.698	0.005
Soil types (S)	1	15.002	0.000	21.389	0.000	17.738	0.000
Depth (D)	1	10.475	0.002	2.805	0.102	3.173	0.082
T × S	1	0.260	0.613	0.732	0.397	0.066	0.799
T × D	1	0.367	0.548	0.105	0.748	0.001	0.982
S × D	1	0.008	0.932	0.890	0.351	1.289	0.263
T × S × D	1	0.006	0.938	0.207	0.652	0.255	0.616

B3. Supplementary figures

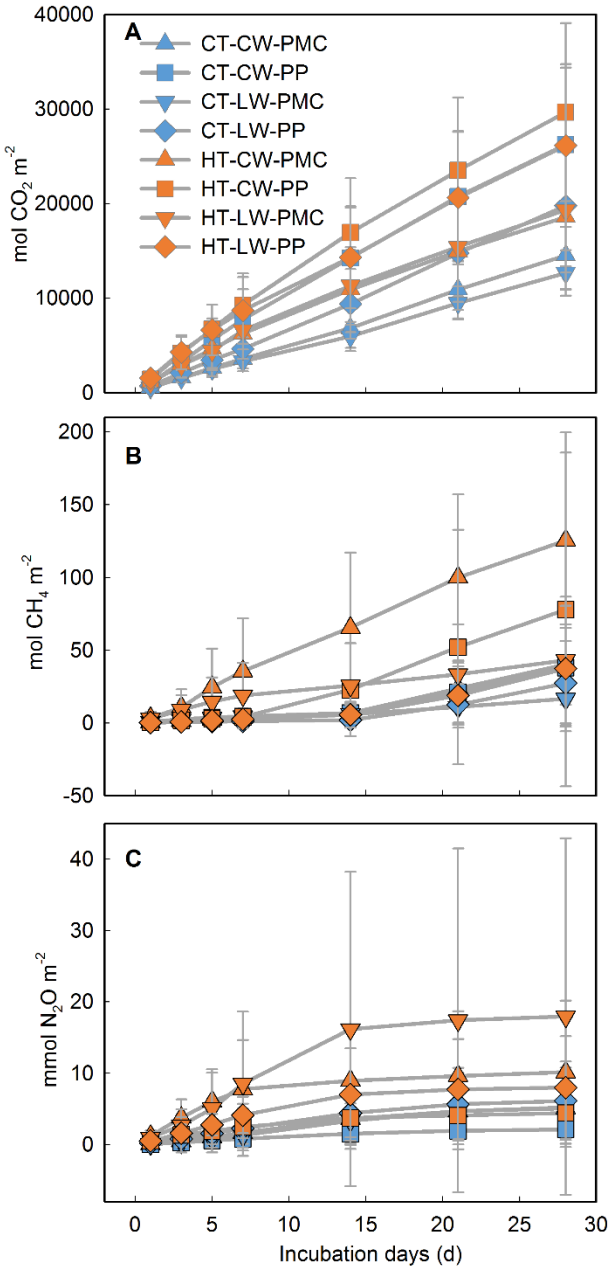

Fig. B.1. Greenhouse gases flux changes over incubation period in two soil types under four temperature-water table treatments during 28-d incubation. A) CO_2 fluxes; B) CH_4 fluxes; C) N_2O fluxes. Treatments: CT: current temperature (15°C), HT: higher temperature (25°C), CW: current water table (25 cm below surface), LW: lower water table (40 cm below surface). Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

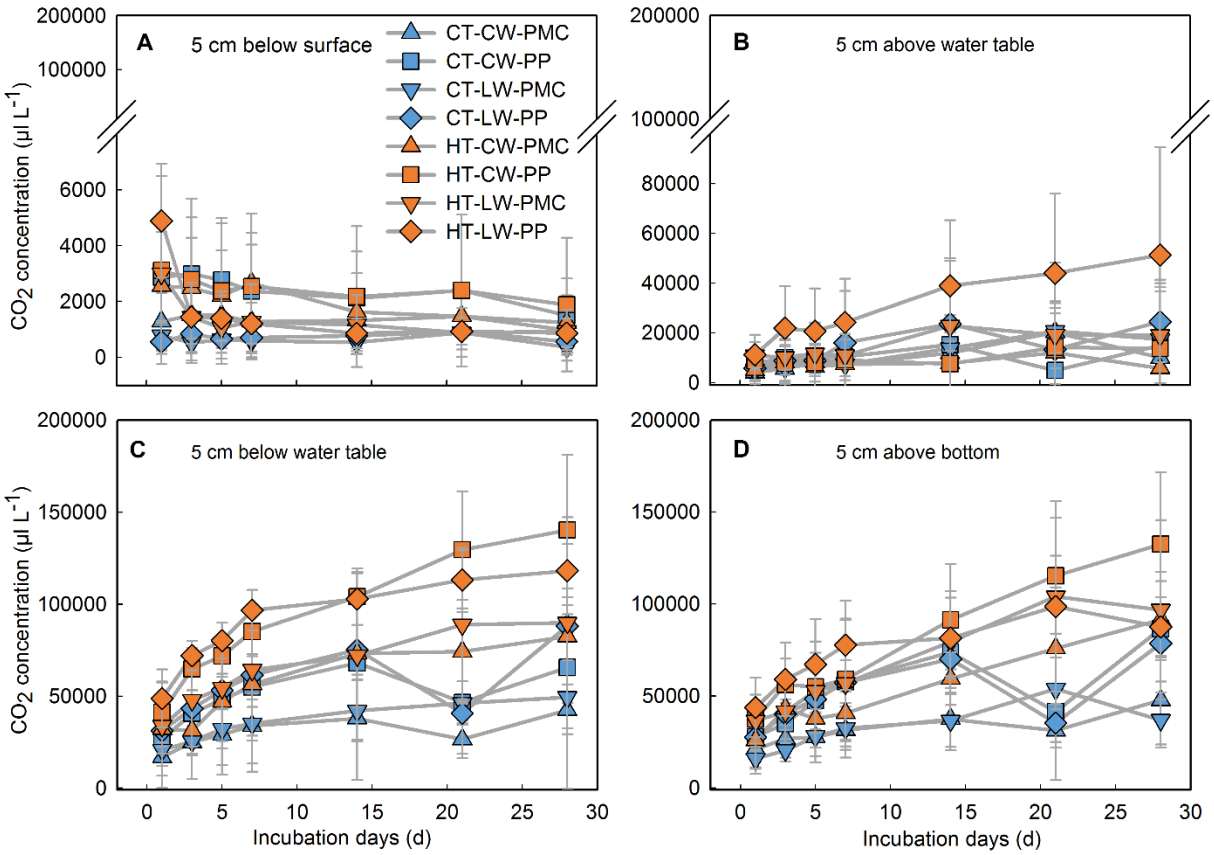

Fig. B.2. Carbon dioxide concentration changes over incubation periods in four different depths in two soil types under four temperature and water table treatments. A) CO_2 concentration in 5 cm below surface; B) CO_2 concentration in 5 cm above water table; C) CO_2 concentration in 5 cm below water table; D) CO_2 concentration in 5 cm above mineral sediments. Treatments: CT: current temperature (15°C), HT: higher temperature (25°C), CW: current water table (25 cm below surface), LW: lower water table (40 cm below surface). Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat. Note: y-axis change in scale.

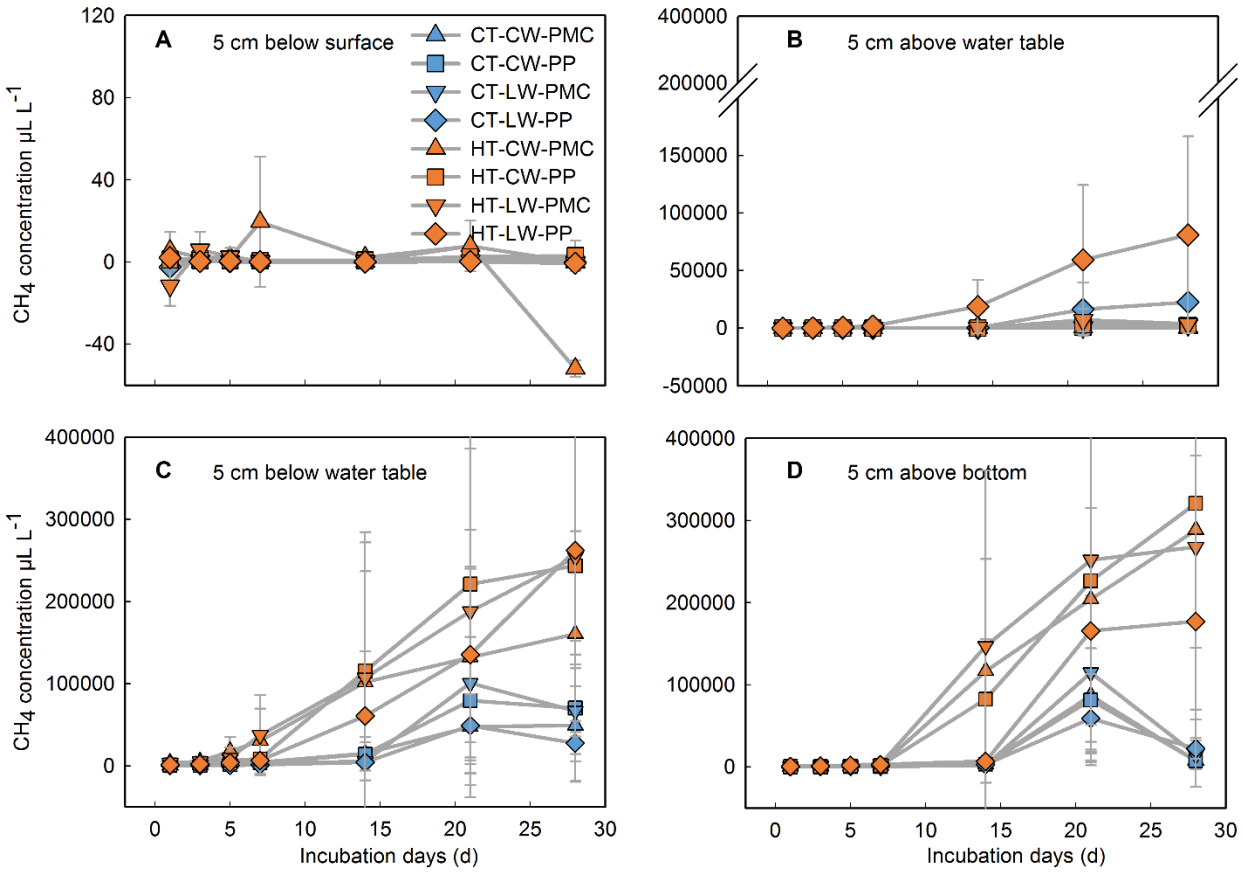

Fig. B.3. Methane concentration changes over incubation periods in four different depths in two soil types under four temperature and water table treatments. A) CH_4 concentration in 5 cm below surface; B) CH_4 concentration in 5 cm above water table; C) CH_4 concentration in 5 cm below water table; D) CH_4 concentration in 5 cm above mineral sediments. Treatments: CT: current temperature (15°C), HT: higher temperature (25°C), CW: current water table (25 cm below surface), LW: lower water table (40 cm below surface). Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat. Note: y-axis change in scale.

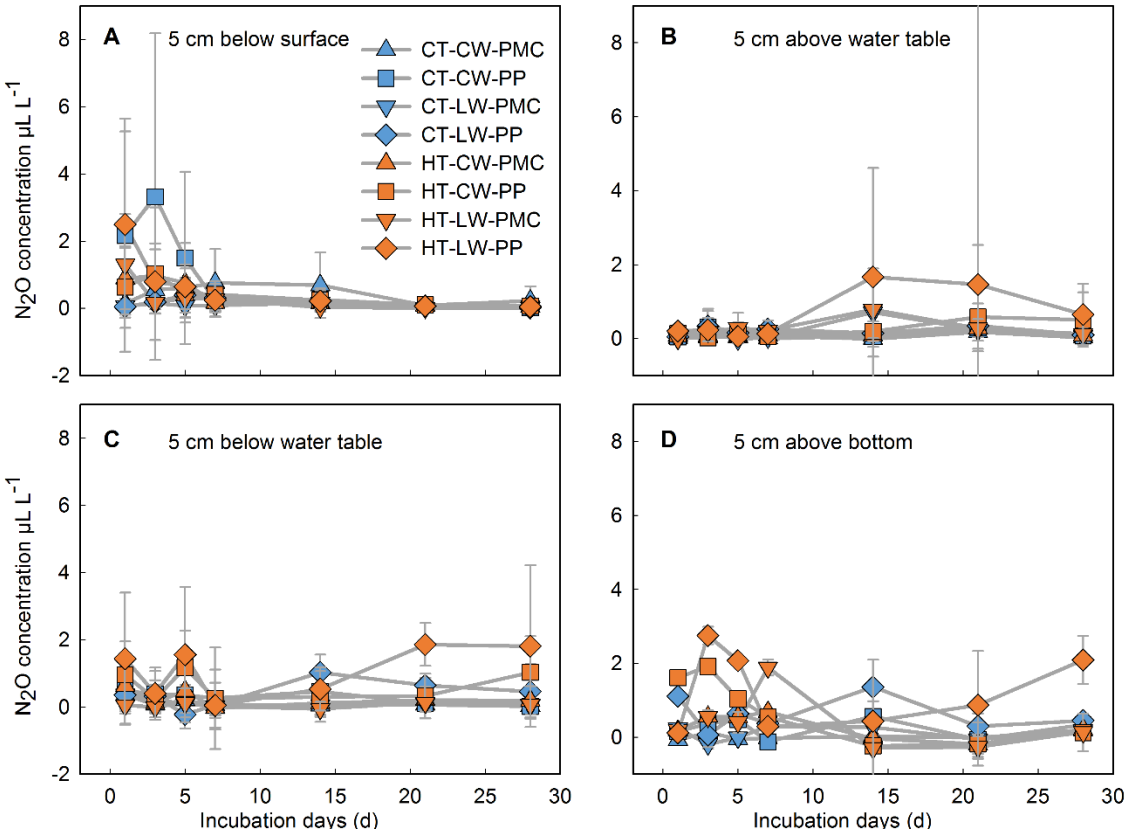

Fig. B.4. Nitrous oxide concentration changes over incubation periods in four different depths in two soil types under four temperature and water table treatments. A) N_2O concentration in 5 cm below surface; B) N_2O concentration in 5 cm above water table; C) N_2O concentration in 5 cm below water table; D) N_2O concentration in 5 cm above mineral sediments. Treatments: CT: current temperature (15°C), HT: higher temperature (25°C), CW: current water table (25 cm below surface), LW: lower water table (40 cm below surface). Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

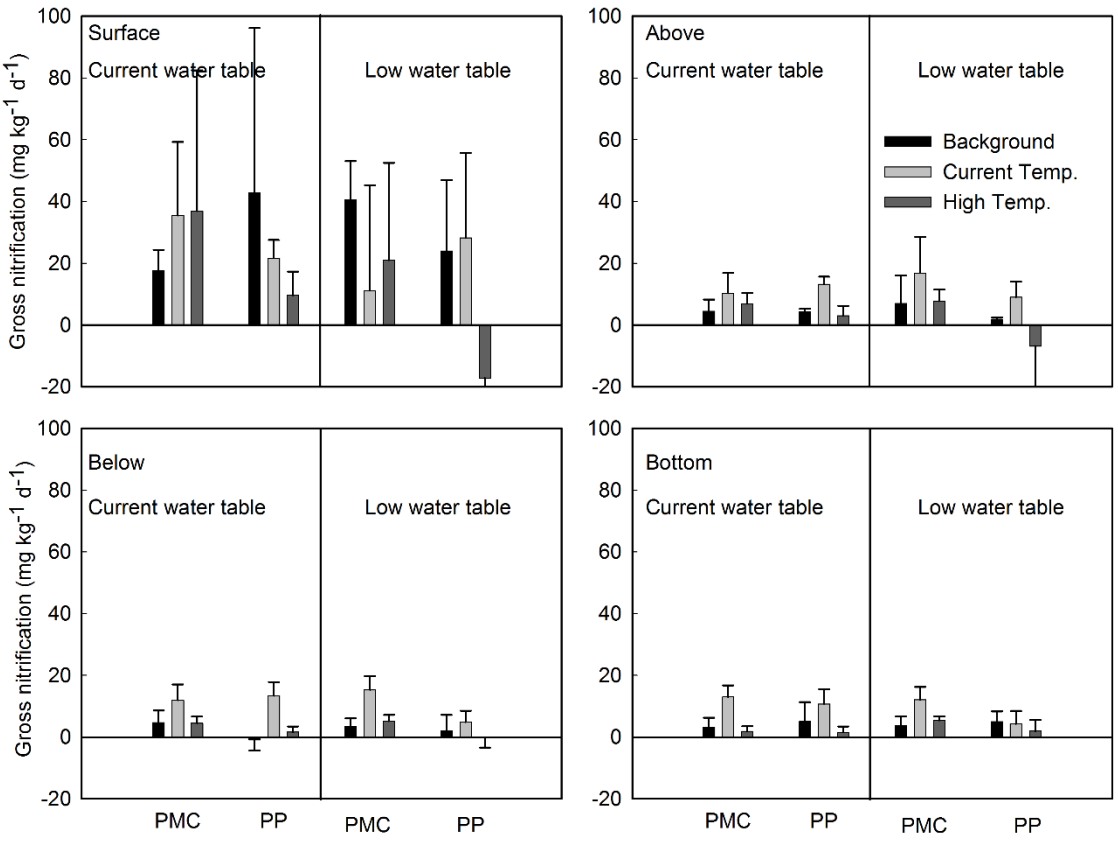

Fig. B.5. Gross nitrification rate over 24 h aerobic incubation in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

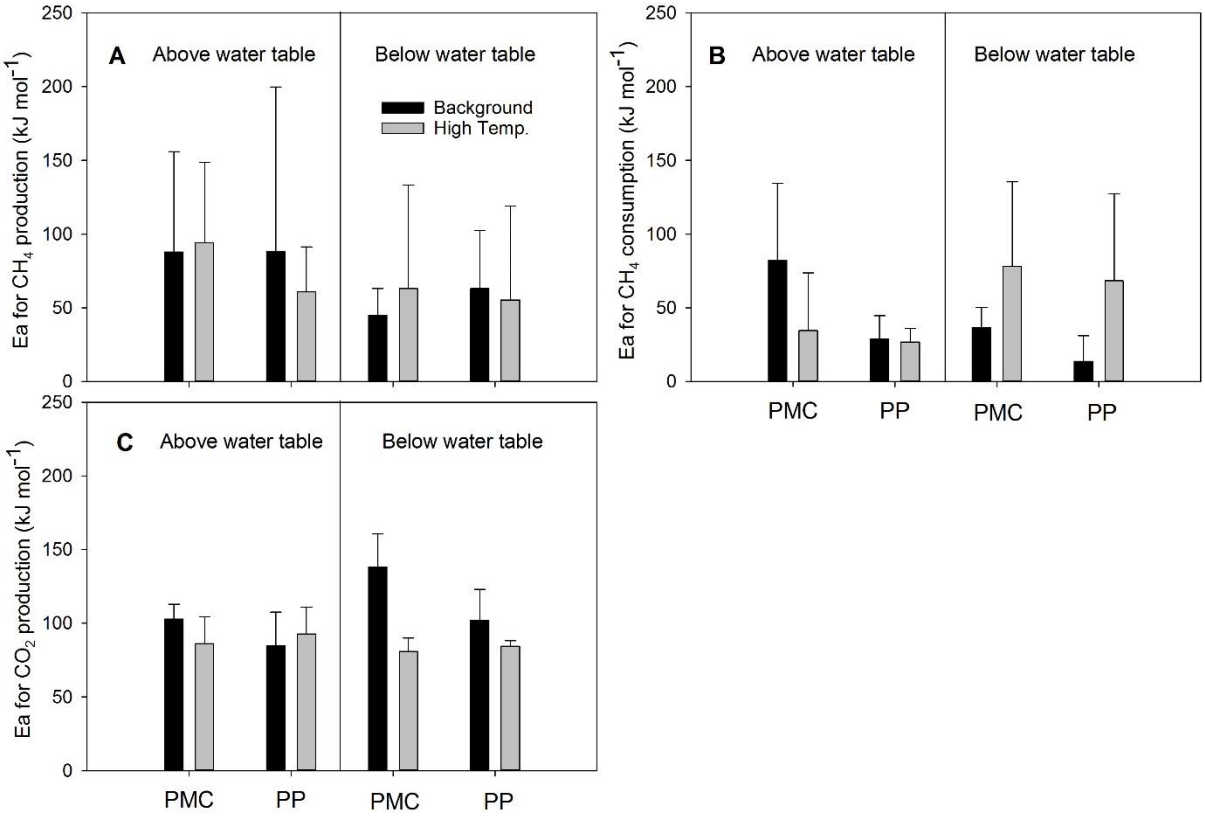

Fig. B.6. Apparent enzyme activation energy (E_a) for CH_4 production and consumption and for CO_2 production from peat 5 cm above and 5 cm below water table with (high temperature) and without (Background) 28d incubation in two soil types. A) E_a for CH_4 production; B) E_a for CH_4 consumption; C) E_a for CO_2 production. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

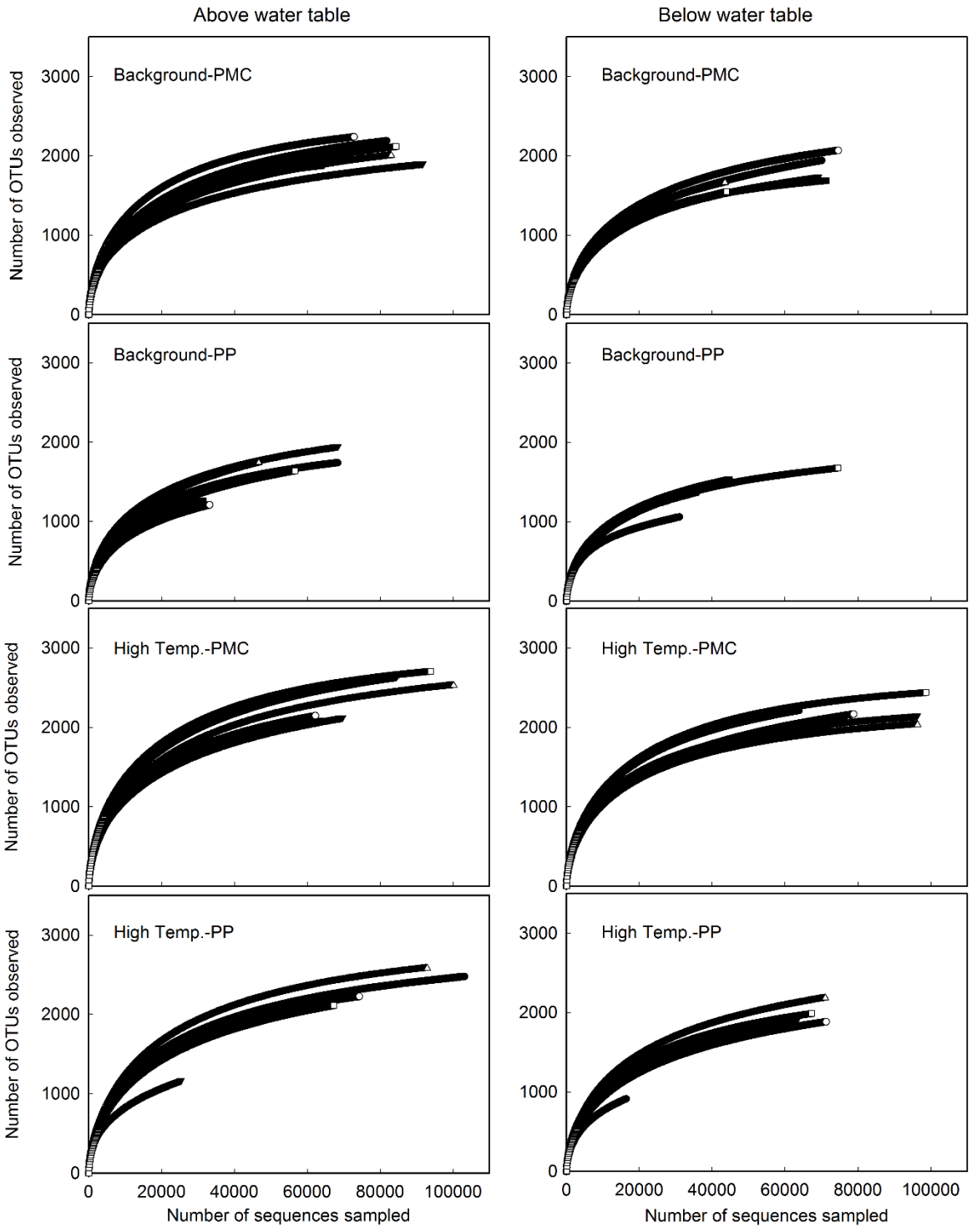

Fig. B.7. Rarefactions of peat samples from peat 5 cm above and 5 cm below water table with (high temperature) and without (Background) 28d incubation in two soil types. Each plot contained six replicates. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

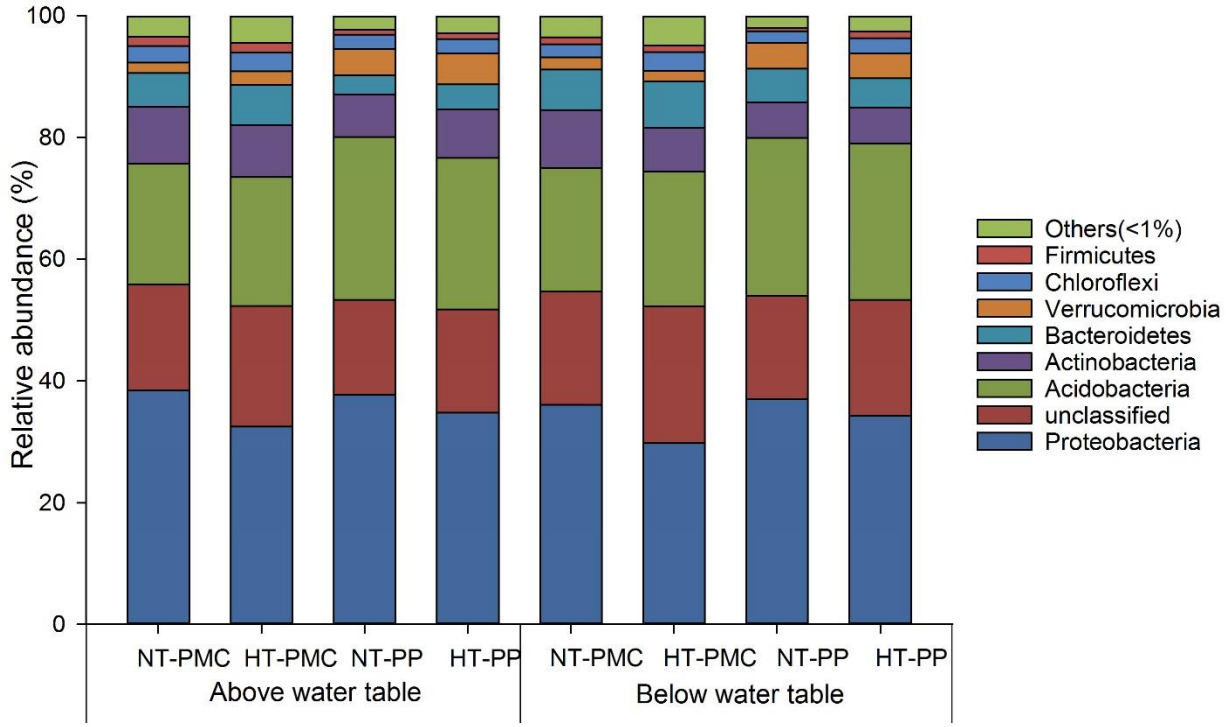

Fig. B.8. Relative abundance of the dominant phyla in from peat 5 cm above and 5 cm below water table with (HT) and without (NT) 28d high temperature incubation in two soil types. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

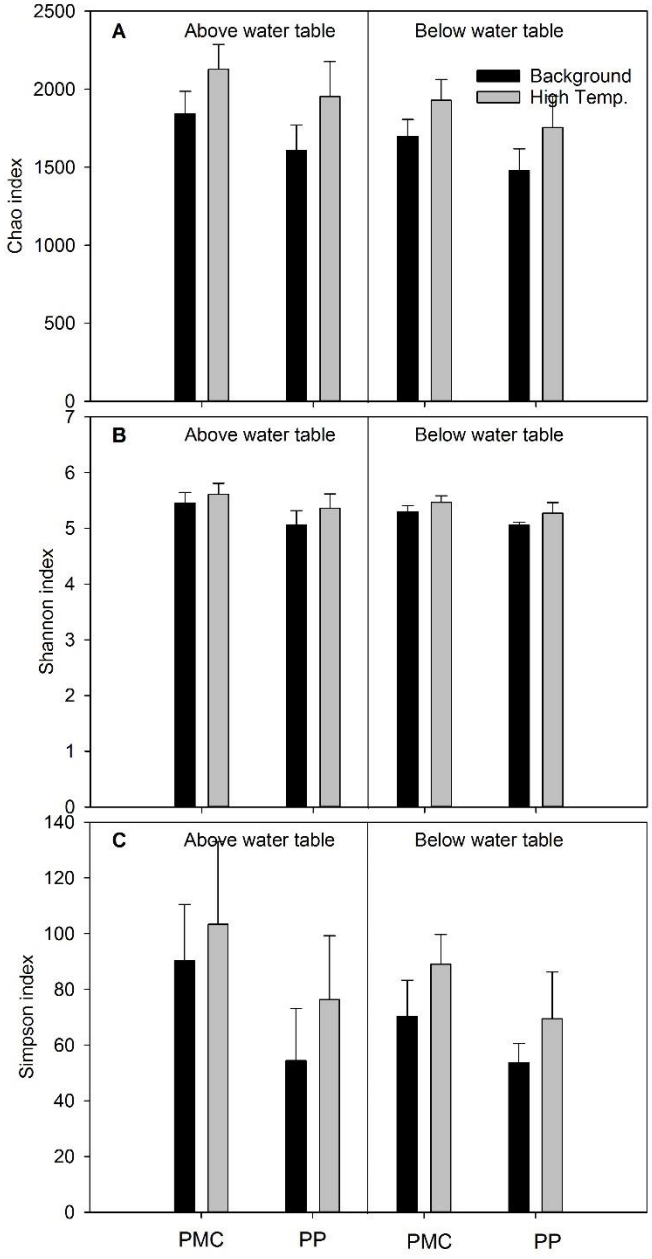

Fig. B.9. Alpha diversity indexes from peat 5 cm above and 5 cm below water table with (high temperature) and without (Background) 28d incubation in two soil types. A) Chao indexes, B) Shannon indexes, C) Simpson indexes. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

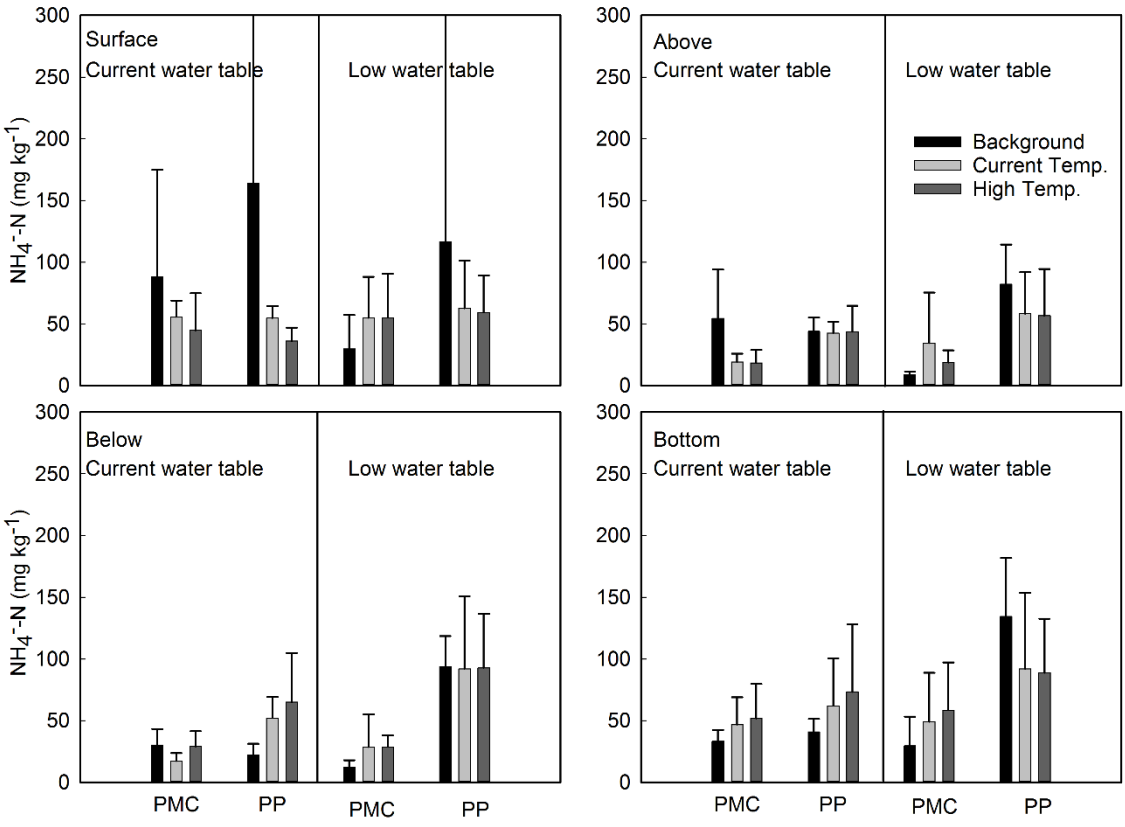

Fig. B.10. Initial [NH_4^+] in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.

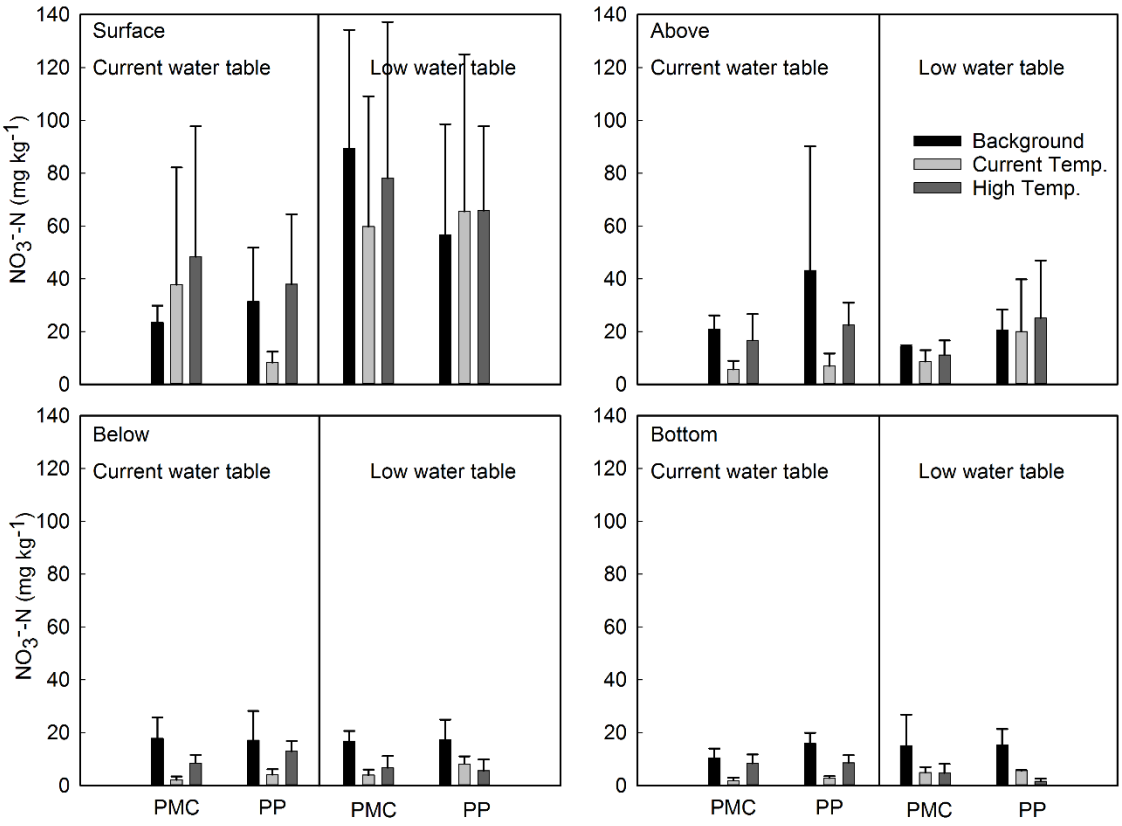

Fig. B.11. Initial [NO_3^-] in peat samples with or without temperature – water table treatment from two soil types. Surface: 0-5 cm from surface, Above: water table to 5 cm above water table, Below: water table to 5 cm below water table, Bottom: bottom to 5 cm above bottom. Soil types: PMC: sedge peat/silty mineral sediments/calcareous sediments, PP: sedge peat/moss peat.